



ANALYTICAL PROFILES OF DRUG SUBSTANCES

Volume 2

Klaus Florey

**Analytical Profiles
of
Drug Substances
Volume 2**

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Analytical Profiles of Drug Substances

Volume 2

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FOREWORD

The concept for gathering together and publishing pertinent information on the physical and chemical properties of various official and new drug substances had its origin with the members of the Section on Pharmaceutical Analysis and Quality Control of the Academy of Pharmaceutical Sciences. More than two years of consideration preceded the authorization of this ambitious project by the Executive Committee of the Academy in the Spring of 1970. The immediate and virtually spontaneous enlistment of the first group of contributors to this work attested to its importance and the wisdom of pursuing its publication.

By coincidence, the delegates to the sesquicentennial anniversary meeting of the United States Pharmacopeial Convention, Inc., in Washington, D.C. on April 8-10, 1970, adopted the following resolution:

Whereas widespread interest has been expressed in the inclusion of additional information about physical and chemical properties of drugs recognized in the United States Pharmacopeia

Be It Resolved that the Board of Trustees consider publishing in the Pharmacopeia, or in a companion publication, information on such attributes as solubilities, pH and pK values, spectra and spectrophotometric constants, and stability data, pertaining to pharmacopeial drugs.

The U.S.P.C. Board of Trustees unanimously approved the resolution in principle on June 4, 1970 and authorized the Director of Revision to include in the U.S.P. monographs such physical-chemical information as he deemed proper and also to cooperate with the Academy of Pharmaceutical Sciences to secure the publication of other physical-chemical data.

It was my privilege to be the President of the Academy during the period when *Analytical Profiles* was under consideration. It is my unusual and unique honor as President of the Academy and Director of U.S.P. Revision to assist in the institution and dedication of this first volume. I trust that it will serve immeasurably in providing the scientific community with an authoritative source of information on the properties of many of our important drug compounds.

January 1971

Thomas J. Macek

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PREFACE

Although the official compendia define a drug substance as to identity, purity, strength, and quality, they normally do not provide other physical or chemical data, nor do they list methods of synthesis or pathways of physical or biological degradation and metabolism. At present such information is scattered through the scientific literature and the files of pharmaceutical laboratories.

For drug substances important enough to be accorded monographs in the official compendia such supplemental information should also be made readily available. To this end the Pharmaceutical Analysis Section, Academy of Pharmaceutical Sciences, has started a cooperative venture to compile and publish *Analytical Profiles of Drug Substances* in a series of volumes of which this is the second. It is also planned to revise and update these profiles at suitable intervals.

Our endeavor has been made possible through the encouragement we have received from many sources and through the enthusiasm and cooperative spirit of our contributors. For coining the term Analytical Profile we are indebted to Dr. James L. Johnson of the Upjohn Company.

We hope that this, our contribution to the better understanding of drug characteristics, will prove to be useful. We welcome new collaborators, and we invite comment and counsel to guide the infant to maturity.

Klaus Florey

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AMPICILLIN

Eugene Ivashkiv

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AMPICILLIN

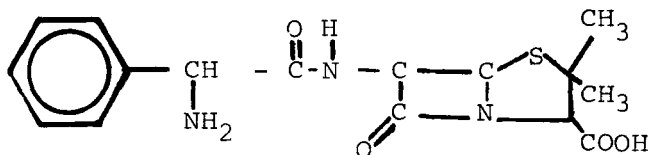
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1. Description

1.1 Name: Ampicillin

Ampicillin^{1,2,3} is designated by Chemical Abstracts as D-(2-amino-2-phenylacetamido)-3,3-dimethyl-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid. Ampicillin is also known as 6/D(-)- α -aminophenylacetamido/ penicillanic acid, D(-)- α -aminobenzylpenicillin⁴ and α -aminobenzylpenicillin⁵.

1.2 Formula and Molecular Weight



$C_{16}H_{19}N_3O_4S$

349.41

1.3 Isomers

The presence of a symmetric C atom in the side chain provides optical isomer⁶. The D-isomer, D(-)- α -aminobenzylpenicillin, is more active than the L-isomer, L(-)- α -aminobenzylpenicillin⁷. The synthesis of ampicillin epimer has been reported^{8,9}.

1.4 Hydrates

It has been reported that ampicillin can exist in anhydrous¹⁰⁻¹³, monohydrate^{14,15} sesquihydrate¹⁶ and trihydrate^{17,18} forms. Austin, et al.¹⁹ postulate that ampicillin exists in anhydrous or trihydrate forms only. Refer to section 2.21.

1.5 Salts

Potassium and sodium salts of ampicillin²⁰⁻²⁹, human lysozyme ampicillin salt³⁰, 2-nitro-1,3-indandione salt³¹, and the ampicillin salt of kanamycin³² have been prepared.

1.6 Appearance, Color and Odor

Ampicillin is a free-flowing, white crystalline powder. It has an odor characteristic of penicillins.

2. Physical Properties

2.1 Spectra

2.11 Infrared Spectra

Substituted monocyclic β -lactams in solution show carbonyl absorption within the range $5.68\text{--}5.78\mu$ ³³. When the β -lactam ring is fused to a thiazolidine ring, the carbonyl absorption occurs in the range $5.62\text{--}5.65\mu$. Morin and co-workers³⁴ have noted rough correspondence between the frequencies of the β -lactam carbonyl absorption and the biological activities of a series of penicillin derivatives.

The infrared spectra of penicillin analogues have been discussed³⁵. The infrared spectrum of ampicillin trihydrate was measured on a Perkin-Elmer Model 21 double-beam spectrophotometer³⁶. The infrared absorption of penicillin derivatives has been recorded and discussed³⁷. Figure 1 and Figure 2 are the spectra of the Squibb Primary Reference Substances of ampicillin trihydrate and anhydrous ampicillin recorded as mineral oil mull and potassium bromide pellets with a Perkin-Elmer Model 21 spectrophotometer. Interpretation of the spectrum of ampicillin trihydrate has been reported³⁸ and is given in Table 1.

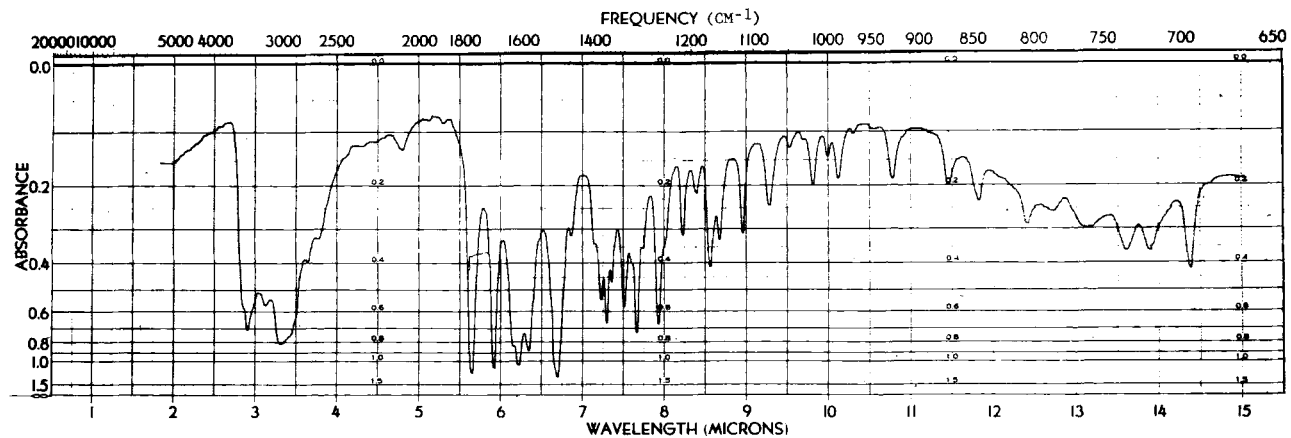


Figure 1. Infrared Spectrum of Ampicillin Trihydrate Squibb Reference Standard.

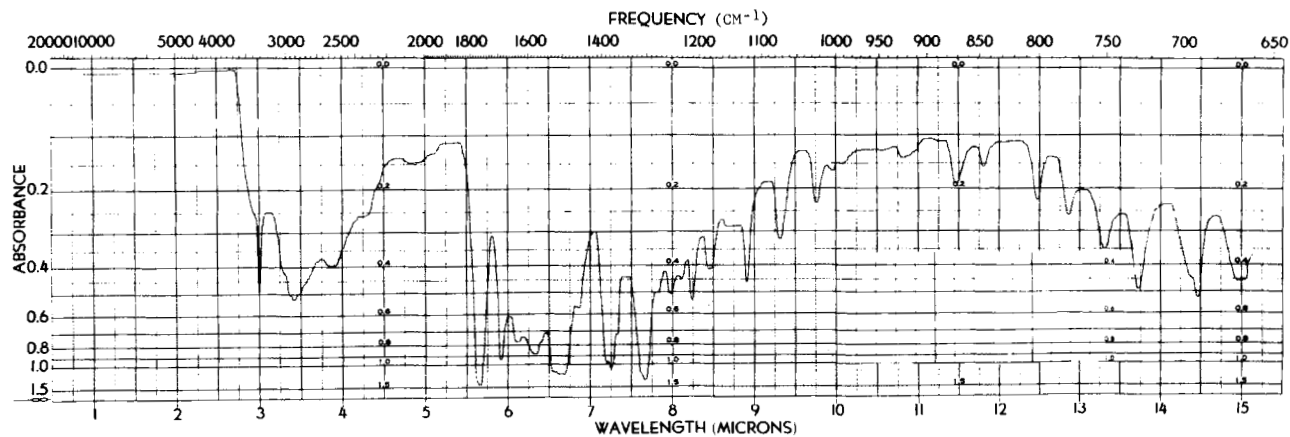


Figure 2. Infrared Spectrum of Anhydrous Ampicillin Squibb Reference Standard.

Table I
Infrared Spectrum of Ampicillin Trihydrate

<u>IR Absorption Band, μ</u>	<u>Interpretation</u>
2.9	H ₂ O
3.1	N'H
weak bands 3.5-4.8	NH ₃ ⁺
5.65	β -lactam C = O
5.90	Amide C = O
6.2 and 6.35	COO ⁻ , NH ₃ ⁺
6.7	Aromatic ring, Amide II, NH ₃ ⁺
14.4	Monosubstituted aromatic ring.

In the solid form, ampicillin trihydrate exists as the zwitterion and the infrared spectrum shows absorptions typical of this type of compound³⁸.

2.12 Nuclear Magnetic Resonance Spectra

NMR spectra of penicillin derivatives in different solvents were recorded on a 100 MHz spectrometer³⁹. Recently, structural studies with ¹³C nuclear magnetic resonance were reported⁴⁰ for penicillins. ¹³C Chemical shift assignments were made for the different carbon atoms. NMR spectra in D₂O solutions of thirteen penicillin derivatives have been recorded and interpreted³⁷. Cohen and Puar⁴¹ studied NMR spectra of ampicillin trihydrate in deuterodimethylsulfoxide (DMSO-d₆) and D₂O-DCl. Tetramethylsilane was used as an internal standard. The Varian XL-100-15 NMR spectrophotometer was used. In the case of DMSO-d₆, β -lactam protons could be easily distinguished by their coupling pattern.

The NMR spectra of the Squibb Primary Reference Substance are given in Fig. 3 and Fig. 4. The assignments are reported in Table II.

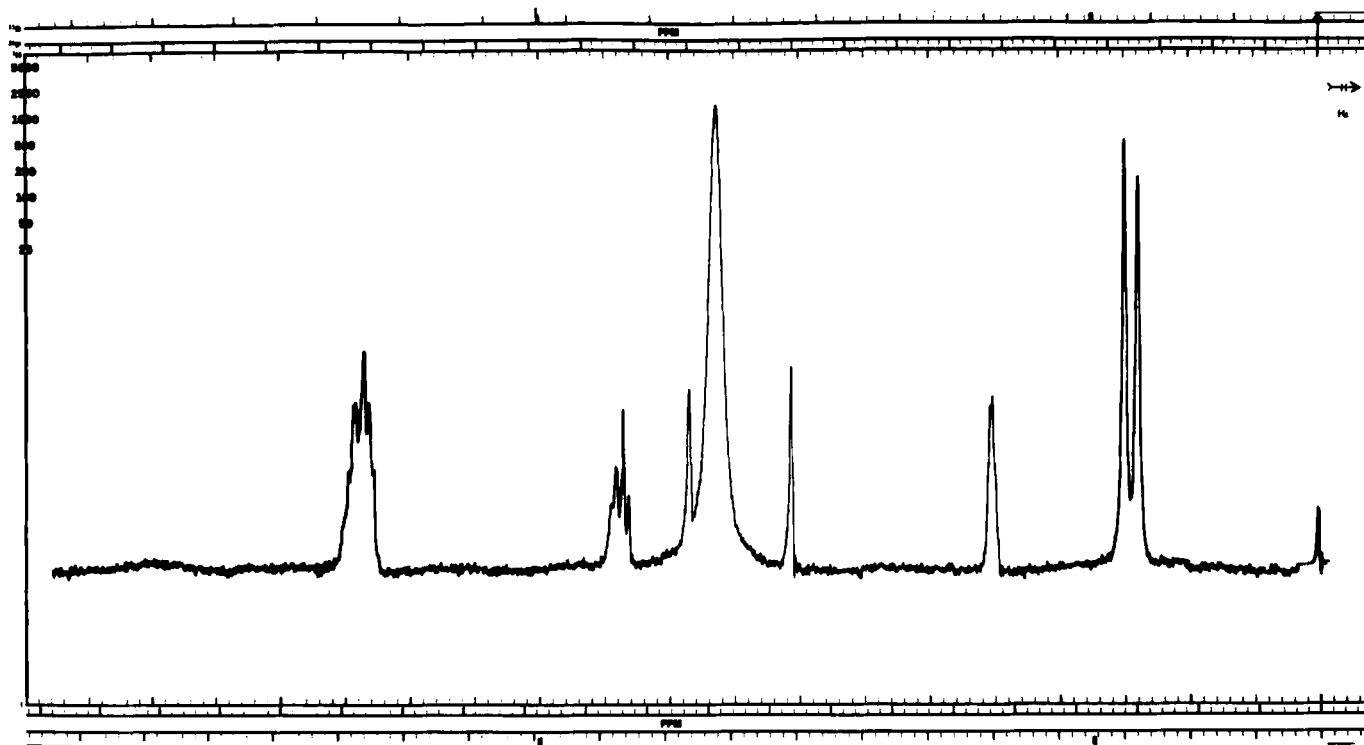


Figure 3. NMR Spectrum of Ampicillin Trihydrate in DMSO-d₆.

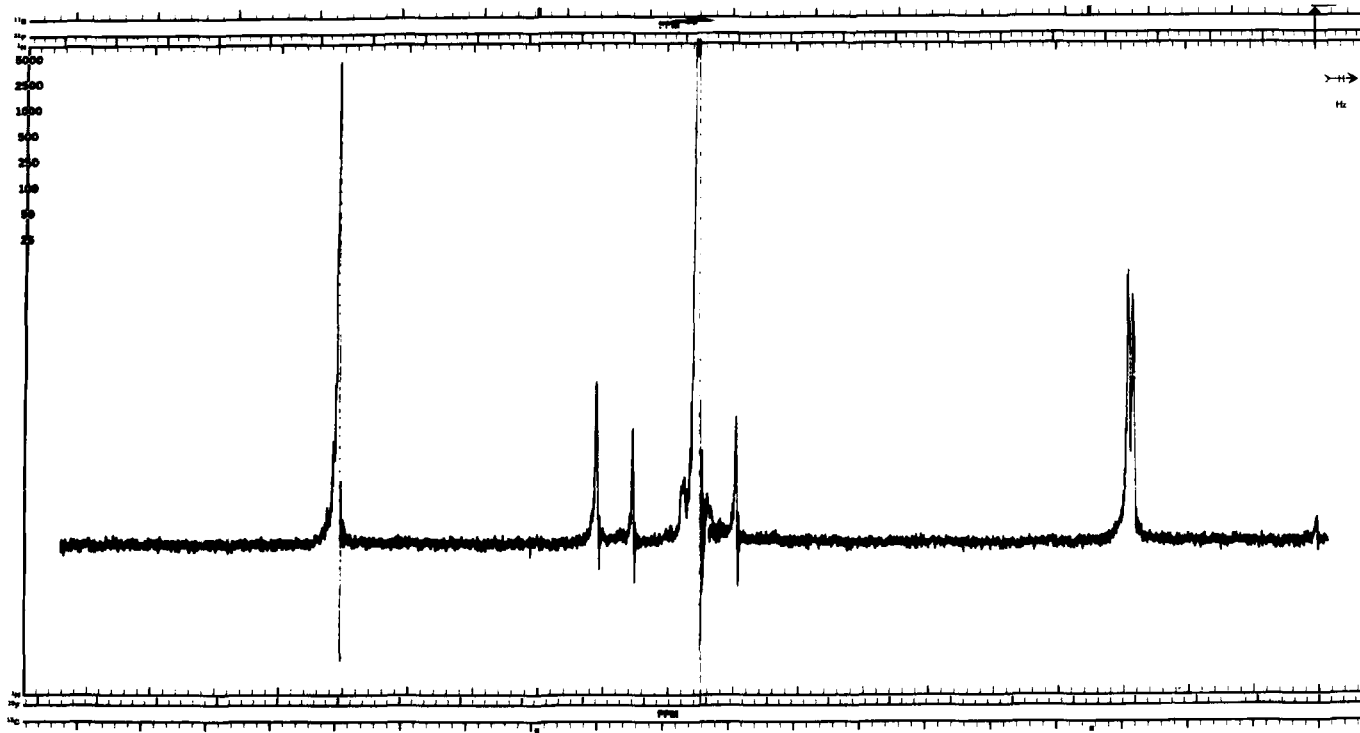
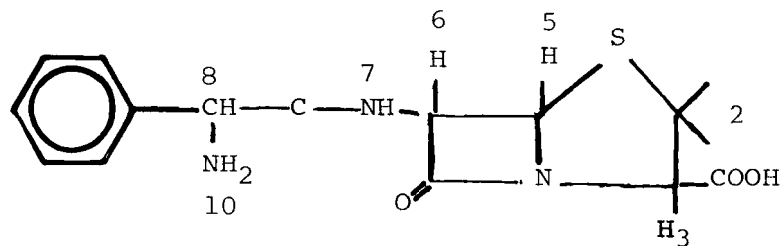


Figure 4. NMR Spectrum of Ampicillin Trihydrate in D_2O -DCI.

Table II
Proton-resonance Lines

Solvent	Chemical Shifts,							
	2H	3H	5H,	6H	7H	8H	9	10
DMSO-d ₆	1.39 1.49	1.39	5.39q 5.39d	(9.0, 4.0) (4.0)	5.95b	4.84	7.36m	4.64b
D ₂ O-DCI	1.41 1.44	4.47	5.54			5.26	7.53	

b = broad, m = multiplet, q = quartet

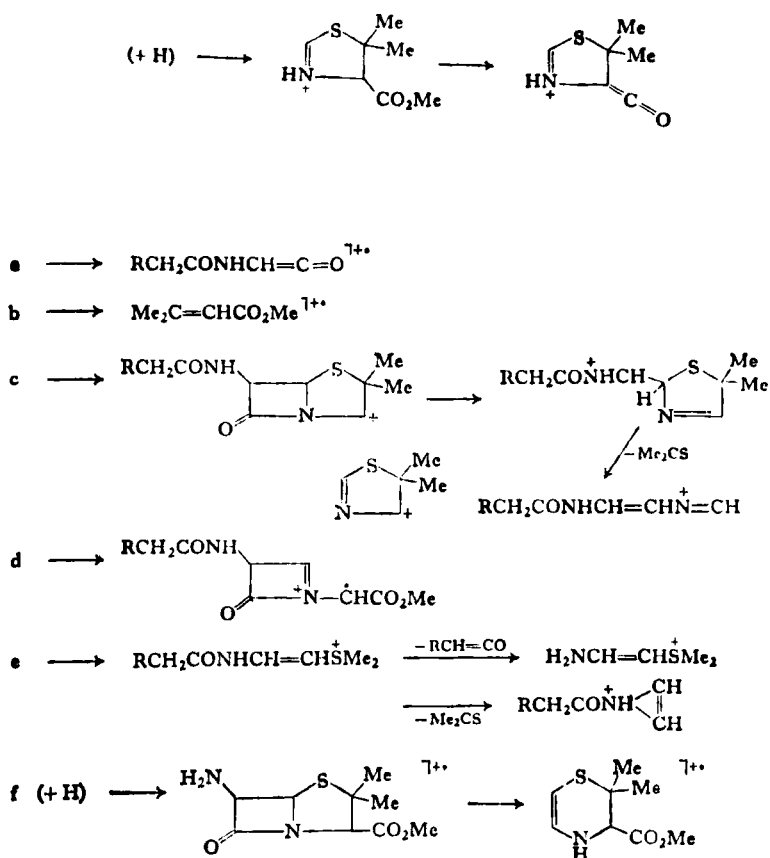


2.13 Mass Spectroscopy

The behavior of the penicillins in the mass spectrometer has been studied by several investigators. The fragmentation of penicillin V has been discussed by Kukolja, et al ⁴². Richter and Biemann⁴³ examined penicillins G and V methyl esters and made numerous accurate mass measurements at high resolution. The nucleus fragmentation by four different routes is shown in Fig.5.

Bochkarev, et al.⁴² studied various penicillin derivatives substituted at the carbonyl group and showed that it was possible to deduce the nature of this substituent from the mass spectrum. The mass spectrum of ampicillin trihydrate was determined by Funke and Cohen⁴⁵ and is shown in Figure 6. They prepared a disilyl derivative using N,O-bis(trimethylsilyl)-acid amide dissolved in pyridine. The mass spectrum exhibits the molecular ion, M^+ , of m/e 493 for the disilyl derivative. The more intense $M^+ - 15$ ion at m/e 478 is due to loss of methyl radical. The m/e 421 ion corresponds to the loss of $Si(CH_3)_2CH_2$ from the molecule or the mono-silyl derivative while the ion 15 amu lower at m/e 406 has lost a methyl group in addition to the silyl function. The diagnostic fragment ion at m/e 178 for silyl derivatives of penicillins demonstrates the presence of 6-aminopenicillanic acid (6-APA) function. In addition, the ion at m/e 106 also is diagnostic for the non-silanized phenylglycyl derivative. The intense ion at m/e 232 is diagnostic for all penicillin derivatives. The mass spectral assignments are summarized in Figure 7.

Figure 5. Penicillin Fragmentation



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EUGENE IVASHKIV

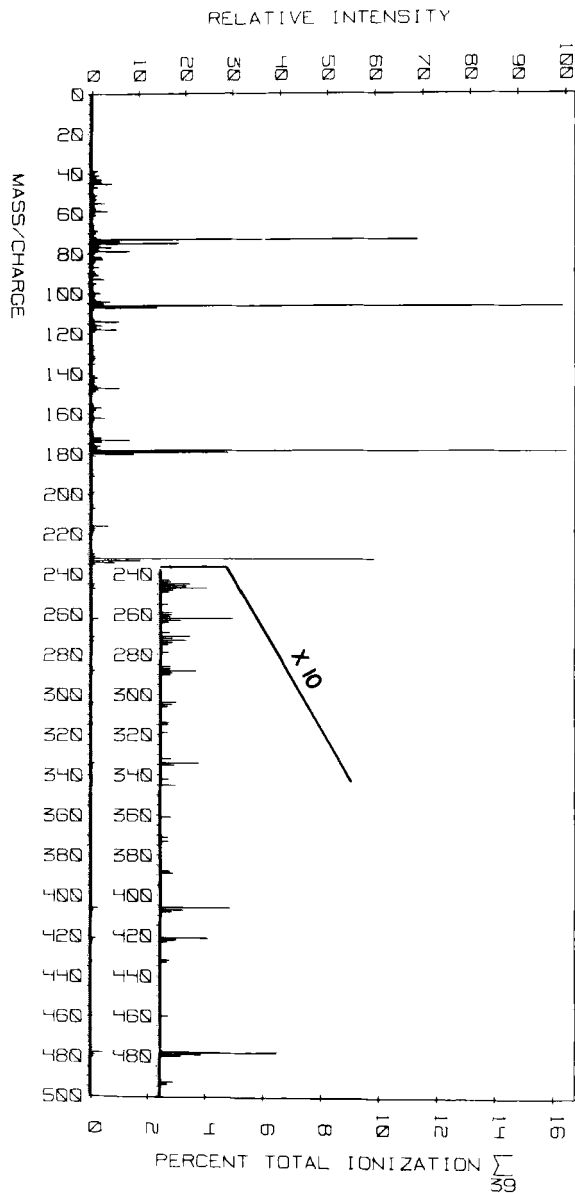


Figure 6. Low Resolution Mass Spectrum of Ampicillin Trihydrate Squibb House Standard.

AMPICILLIN

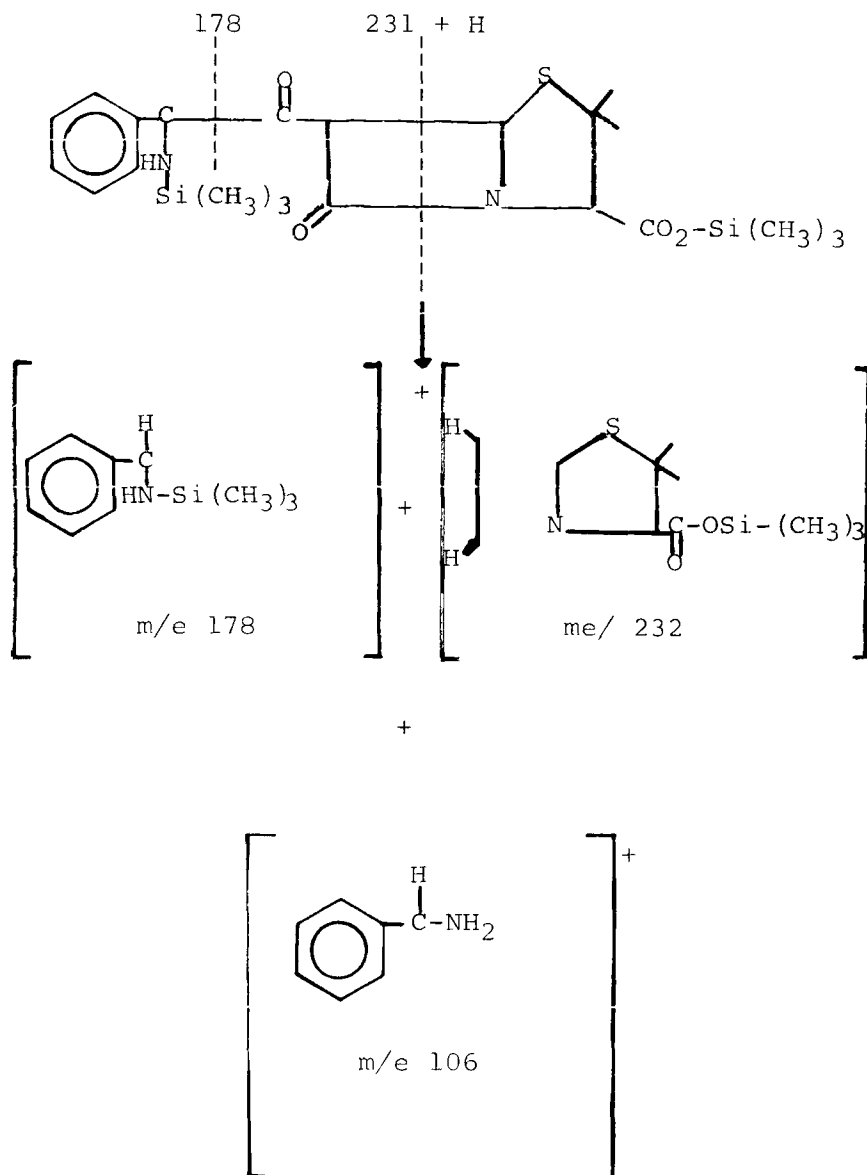


Fig. 7. Fragmentation of Ampicillin Derivative.

2.14 Ultraviolet Absorption

Ultraviolet spectra of ampicillin trihydrate, Squibb Primary Reference Substance were recorded on a Cary Model 15 spectrophotometer⁴⁶ and $E_{1\%}^{1\text{ cm}}$ values calculated. The results are given in Table III.

Table III

Ultraviolet Absorption
of Ampicillin Trihydrate

	<u>Wavelength, nm</u>		
<u>Phosphate buffer pH 5.3</u>	257	262	268
$E_{1\%}^{1\text{ cm}}$	8.69	7.81	5.61
<u>Phosphate buffer pH 7.0</u>	257	262	268
$E_{1\%}^{1\text{ cm}}$	7.94	6.69	4.55
<u>Phosphate buffer 9.5</u>	258	268	-
$E_{1\%}^{1\text{ cm}}$	7.28	5.03	

With the increase of pH of buffer solution $E_{1\%}^{1\text{ cm}}$ decreases. No peak at 268 nm in pH 9.5 buffer was obtained.

Saturated methanolic ampicillin trihydrate solutions produced maximum absorption at 258, 262 and 268 nm⁴⁶.

2.2 Crystal Properties

2.21 Crystalline Modification of Ampicillin

Ampicillin crystallizes into two forms depending on the temperature of the aqueous solution¹⁹. Anhydrous ampicillin is obtained from aqueous solutions at temperatures above 60°C; the trihydrate is obtained from aqueous solutions at temperatures below 50°C. The existence of anhydrous, monohydrate, sesquihydrate and trihydrate forms of ampicillin was claimed¹⁰⁻¹⁸. It has been shown by x-ray diffraction⁴⁷ that ampicillin monohydrate existed. Austin *et.al.*¹⁹ concluded from a study of the infrared spectra of various forms of crystalline ampicillin that only the anhydrate and trihydrate exist. They believed the other hydrates were either amorphous or partially dehydrated trihydrate⁴⁸. Crystals of anhydrous and trihydrate ampicillin were prepared. James and Hall reported crystallographic data for ampicillin trihydrate. They showed that ampicillin exists as a zwitterion with three water molecules extensively involved with hydrogen bonding. Grant and Alburn⁴⁷ have distinguished between a crystalline anhydrous form and a monohydrate, which differ in solid-state infrared spectra, density, solubility and thermal stability. Anhydrous ampicillin was prepared from any form of ampicillin but reasonably rapid conversion of trihydrate required a temperature 80°-100°C¹⁹. Hydrated ampicillin was also converted into an anhydrate by heating a suspension in nitromethane or other nitrohydrocarbons.

2.22 X-ray Diffraction

Single-crystal x-ray diffraction was used to deduce the structure of benzylpenicillin⁴⁹. Several salts of benzyl penicillin was analyzed and bond lengths determined⁵⁰. X-ray diffraction analysis of anhydrous ampicillin and ampicillin monohydrate were performed by T.Doyne and reported⁴⁷. Fig. 8 shows the x-ray diffraction of ampicillin and gives the d values characteristic of this crystal form of ampicillin trihydrate⁵¹.

2.23 Melting Range

Ampicillin monohydrate melts with decomposition at 202°C and sodium ampicillin at 205°C¹⁴, sesquihydrate and anhydrous ampicillin decompose at 199-202°C¹⁶. The melting range for ampicillin trihydrate with decomposition at 214.5° - 215.5°C was reported⁵² and melting with decomposition has been reported at 202°- 204°C by another investigator⁵³.

2.24 Differential Thermal Analysis

Jacobson⁵⁴ recorded differential thermal analysis curves of ampicillin trihydrate, Squibb Primary Reference Substance, on a DuPont Differential Thermal Analyzer with a temperature rise of 15° per minute. Endothermic steps at 88°, 92°, and a large endotherm at 100°C were observed. Some samples of ampicillin trihydrate had a single large endotherm at 125°C while others had the large endotherm at about 105°- 110°C⁵⁴. Differential thermal analysis for the control of phase transformation of ampicillin is used⁵⁵. It has been found that thermograms were reproducible for a given lot of ampicillin trihydrate but varied from lot to lot. The phase transformation of ampicillin trihydrate by the differential thermal analysis under pressure was studied⁵⁶.

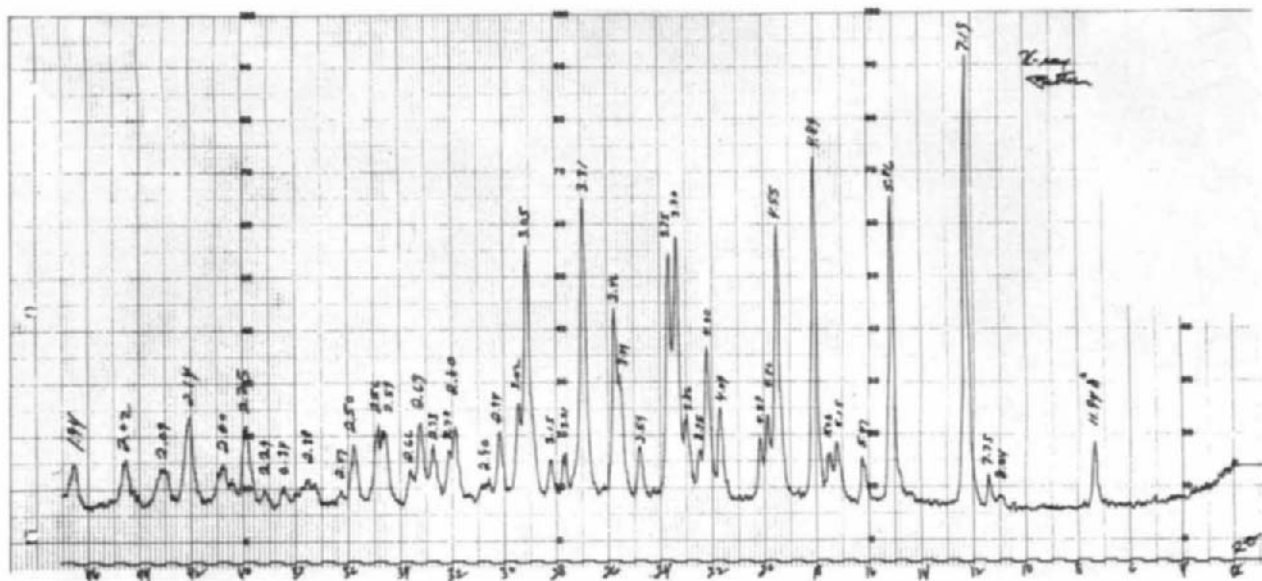


Figure 8. X-Ray Diffraction Pattern of Ampicillin Trihydrate Squibb Sample, Lot 18.

2.25 Thermal Gravimetric Analysis

A DuPont Thermogravimetric Analyzer, Model 950, indicated total volatile material to be 13.8% in ampicillin trihydrate Squibb Primary Reference Substance⁵⁴. The theory is 13.39%.

2.3 Solubility

The solubilities of anhydrous ampicillin, ampicillin trihydrate and sodium ampicillin from two manufacturers in various solvents were determined by Marsh and Weiss⁵⁷. They found a variation in the solubility of sodium ampicillin from two different manufacturers and indicated this could be due to crystalline structure. The results are reported in Table IV.

Anhydrous ampicillin and ampicillin trihydrate were compared for solubility in distilled water at temperatures ranging from 7.5° to 50°C⁵⁸.

2.4 Ionization Constant, pK

Rapson and Bird⁵⁹ reported ionization constants for ampicillin to be: $pK_1 = 2.53 \pm 0.004$ and $pK_2 = 7.24 \pm 0.02$. Jacobson and Russo-Alesi⁶⁰ calculated pK_2 for ampicillin trihydrate to be 7.24. Hou and Poole⁶¹ reported $pK_1 = 2.66 \pm 0.03$ and $pK_2 = 7.24 \pm 0.03$.

2.5 Optical Rotation

	<u>Reference</u>
Ampicillin monohydrate $[\alpha]_D^{21} + 281^\circ$ (C = 1 in H ₂ O)	14
Ampicillin sesquihydrate $[\alpha]_D^{20} + 283.1^\circ$ (C = 1 in H ₂ O)	16
Sodium Ampicillin $[\alpha]_D^{20} + 209^\circ$ (C = 0.2 in H ₂ O)	14
Anhydrous Ampicillin $[\alpha]_D^{20} + 287.9^\circ$ (C = 1 in H ₂ O)	16

Table IV
Solubility of Ampicillin (mg/ml)

<u>Solvent</u>	<u>Ampicillin</u>			<u>Trihydrate</u>
	<u>Anhydrous</u>	<u>Na Salt I*</u>	<u>Na Salt II*</u>	
Water	10.098	> 20	> 20	7.558
Methanol	2.968	> 20	> 20	6.649
Ethanol	0.390	> 20	19.780	2.538
Isopropanol	0.055	1.13	6.405	-
Isoamyl alcohol	0.125	1.902	19.300	-
Cyclohexane	0.048	0.075	0.0	0.068
Benzene	0.002	0.022	0.0	0.032
Petroleum Ether	0.010	0.025	0.0	0.038
Isooctane	0.0	0.022	0.0	0.022
Carbon Tetrachloride	0.008	0.032	0.0	0.025
Ethyl Acetate	0.025	0.035	0.058	0.225
Isoamyl Acetate	0.030	0.105	0.048	0.078
Acetone	0.125	0.518	> 20	8.952
Methyl Ethyl Ketone	0.052	0.178	> 20	2.790
Diethyl Ether	0.022	0.022	0.0	0.03
Ethylene Chloride	0.032	0.060	0.032	0.068
1,4-Dioxane	0.595	1.375	1.845	2.772
Chloroform	0.095	0.118	0.155	0.075

continued...

AMPICILLIN

Table IV. Solubility of Ampicillin (mg/ml), continued

<u>Solvent</u>	<u>Anhydrous</u>	<u>Ampicillin</u>		<u>Trihydrate</u>
		<u>Na Salt I[*]</u>	<u>Na Salt II[*]</u>	
Carbon Disulfide	0.015	0.010	0.0	0.022
Pyridine	2.100	3.256	20	12.131
Formamide	20	20	20	20
Ethylene Glycol	18.415	20	20	19.128
Propylene Glycol	2.230	20	20	4.138
Dimethylsulfoxide	20	20	20	20
0.1N NaOH	20	20	20	20
0.1N HCl	20	20	20	20

* Sodium ampicillin from two different manufacturers.

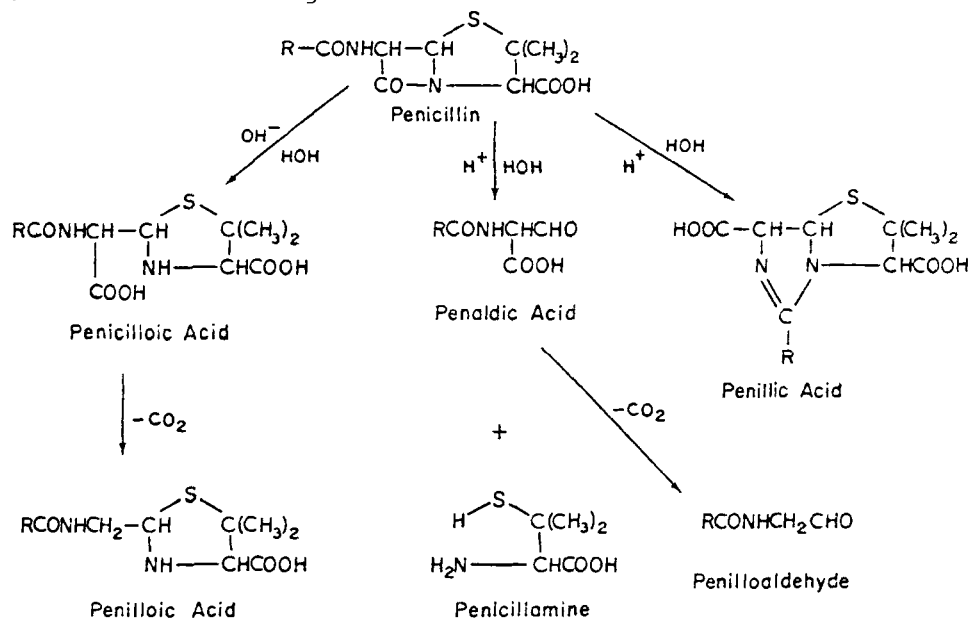
The optical rotation for ampicillin trihydrate, $[\alpha]_D^{26} + 245.10$ ($C = 1$, buffer pH 8.0) and ampicillin anhydrous, $[\alpha]_D^{26} + 289^\circ$ ($C = 1$, buffer pH 8.0) has been reported⁶². Dursch⁶³ reported the optical rotation for ampicillin trihydrate as $[\alpha]_D^{27} + 249.5^\circ$ to 252.7° ($C = 1$, buffer pH 8.0). Optical rotation in 2N hydrochloric acid, after dissolution of 1% ampicillin and 60 minutes of standing at 25°C is $[\alpha]_D^{25} = -85.5^\circ\text{C}$ ⁶⁴.

3. Ampicillin Stability

3.1 Modes of Penicillin Degradation

The main course of deterioration penicillins is hydrolysis. The course of the hydrolysis is shown in Figure 9. Dunham⁶⁵ reviewed modes of penicillin degradation.

Figure 9. Penicillin Degradation



Reproduced with permission from "Textbook of Organic Medicinal and Pharmaceutical Chemistry", edited by C.O. Wilson, O. Gisvol and R.F. Doerge, 5th Ed., 1966. Lippincott Co., Philadelphia and Toronto.

3.2 Stability of Ampicillin in Solutions

Savello and Shangraw⁶⁶ showed that freezing sodium ampicillin solutions at -200 to -78°C generally increased the degradation of 1% solutions and decreased the degradation in 25% solutions. The concentration of ampicillin in solution and the type of vehicle control degradation. Dextrose and mannitol act as catalysts in the hydrolysis of ampicillin. Ampicillin at a 1% concentration is incompatible with dextrose. Normal saline is the most suitable vehicle for intravenous administration of ampicillin. Gallelli⁶⁷ claimed that sodium ampicillin is stable at 5°C and 25°C in 1% sodium chloride solutions also containing 5% dextrose. Jacobs *et al.*,⁶⁸ studied the stability of ampicillin in 5% dextrose, 4.3% dextrose, 0.18% sodium chloride, 0.9% sodium chloride, Hartmann's solution and $1/6\text{ M}$ sodium lactate. Ampicillin was least stable in solutions containing lactate. Ampicillin was least stable in solutions containing lactate ion, losing 40% activity after 4 hours and more than 60% after 24 hours. Ampicillin is inactivated by sucrose, dextrose and dextran solutions at alkaline pH⁶⁹. The stability of ampicillin in 0.2M acetate, citrate, phosphate and lactate buffers at pH 4.5⁷⁰ and in solutions at pH 6 and pH 6.5 was studied⁷⁷. The decomposition rate of ampicillin in a 1% solution follows first order kinetics and obeys the Arrhenius equation when compared at 40°C , 50°C , and 60°C . These findings were confirmed⁷² when the decomposition rate was studied in the presence of H^{+} ions. Degradation of ampicillin is second order in the presence of OH^{-} ions. The stability of ampicillin in aqueous solutions at pH 4 - 9 was determined⁷³. The kinetics of degradation of ampicillin in solutions were in-

vestigated at 35°C and constant ionic strength of 0.5 over a pH range of 0.8 to 10⁷⁴. The observed rates were first-order and significantly influenced by general acid and general base catalysis. The apparent heats of activation for ampicillin degradation in solutions were 16.4, 18.3, and 9.2 k cal/mole in buffers of pH 1.35, 4.93 and 9.78 respectively. The pH - rate profile in buffer solutions showed a minimum at a pH of 4.85. However, at zero buffer concentration the maximum stability was shifted to a pH of 5.85. Ampicillin was stable in solutions at pH 3 - 9 when stored for 24 hours at 5°C and 25°C. Ampicillin was unstable in solutions at pH 10 when stored at above conditions⁷⁵. Bundgaard⁷⁸ demonstrated a metastable intermediate in isomerization of penicillin to penicillenic acid in aqueous solutions. The degradation of ampicillin trihydrate in solution is greatly influenced not only by pH, but also by the types of buffer salts used^{74,77}. 2-Amino-2 (hydroxymethyl)-1,3-propanediol (Tris) buffer of pH 7 is highly deleterious to the stability of ampicillin, but not at pH 5.0. Citrate presents a converse pattern in that ampicillin is relatively stable at pH 7 but not so at pH 5. Phosphate is intermediate in action but tends to resemble the Tris pattern. Jacobson and Russo-Alesi⁷⁸ prepared ampicillin trihydrate solutions at a concentration of 5 mg/ml by dissolving it in buffer solutions of pH 5.0 or pH 7.0 prepared from 0.1M sodium acetate and acetic acid and in buffers of pH 8.0 or pH 8.8 prepared from 0.1M 2-amino-2 (hydroxy methyl)-1, 3-propanediol and acetic acid. Solutions were kept at room temperature and were analyzed by iodometric titration. Stability data is given in Table V.

Table V
Stability of Ampicillin as Function of pH

<u>Time, hours</u>	<u>Percentage Activity Remaining</u>			
	<u>pH 5</u>	<u>pH 7</u>	<u>pH 8</u>	<u>pH 8.8</u>
0.5	-	-	96.0	81.9
1	-	-	87.3	65.8
2	99.4	100.6	73.3	43.6
3	-	-	62.0	28.9
4	100.6	100.0	54.0	17.4
5	-	-	44.0	11.4
24	92.0	100.3	-	-
27	-	-	0	0
48	92.0	96.5	-	-
96	83.0	91	-	-

AMPICILLIN

George⁷⁹ studied the stability of dilute ampicillin solutions at pH 5.8-6.0 and stored at 5°C. Results from microbiological assays are reported in Table VI.

Table VI

Stability of Ampicillin
in Dilute Phosphate Solutions

Ampicillin, mcg/ml	Ampicillin Left, %		
	Days		
	6	8	14
3	102	-	80
6	97	102	88
10	98	99	91
15	99	99	92
25	102	-	-
40	100	-	-

The stability of ampicillin in buffers prepared from 0.1 M Tris buffer and acetic acid as a function of pH and temperature has been studied⁸⁰. Solutions containing 5 mg per ml of ampicillin were made and stored at room temperature or 5°C for 29 days. Solutions were tested for the remaining ampicillin by the colorimetric hydroxylamine method. Results are reported in Table VII.

Table VII
Stability of Ampicillin as Function of pH and Temperature

		<u>Percent Activity Remaining at Room Temperature</u>						
<u>Time in Days</u>	<u>pH 8.4</u>	<u>7.5</u>	<u>6.4</u>	<u>4.9</u>	<u>3.8</u>	<u>2.9</u>	<u>2.3</u>	<u>1.7</u>
1	0	16.5	53.1	90.6	89.4	73.1	47.9	24.7
2	0	1.8	32.2	82.1	88.7	54.2	26.7	8.0
6	0	0	4.8	60.1	67.6	21.7	3.5	0
9	0	0	0	45.2	54.4	10.7	1.1	0
15	0	0	0	26.5	39.5	3.9	0	0
29	0	0	0	11.4	13.9	1	0	0
		<u>Percent Activity Remaining at 5°C</u>						
<u>Time in Days</u>	<u>pH 8.4</u>	<u>7.5</u>	<u>6.4</u>	<u>4.9</u>	<u>3.8</u>	<u>2.9</u>	<u>2.3</u>	<u>1.7</u>
1	43.9	81.4	92.7	100	95.2	94.2	85.3	83.0
2	23.1	71.2	87.6	93.4	95.5	92.1	81.6	67.7
6	1	42.5	78.9	94.6	86.3	82.4	64.7	39.8
9	0	27.7	73.6	93.4	91.9	71.5	51.2	23.7
15	0	11.9	63.5	85.1	89.9	58.6	33.2	9.9
29	0	0	48.6	79.9	86.3	39.8	13.6	1.5

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The stability of ampicillin trihydrate in sodium acetate and Tris buffers as a function of time was studied⁸¹. Data is reported in Table VIII.

Table VIII
Stability of Ampicillin Trihydrate in Sodium Acetate and
Tris Buffers as a Function of pH

(Ampicillin (2 mg/ml, Hydroxylamine method)

	<u>Initial pH</u>	<u>5 Hours</u>	<u>24 Hours</u>	<u>48 Hours</u>	<u>120 Hours</u>	<u>216 Hours</u>	<u>312 Hours</u>	<u>Final pH</u>
0.1M Tris	4.9	98.1	93.9	92.4	73.4	57.4	40.8	4.8
0.1M NaOAc	4.9	98.3	94.3	94.7	78.9	65.0	49.9	4.9
0.1M Tris	5.9	98.8	90.1	91.5	66.9	45.6	30.5	5.6
0.1M NaOAc	6.0	102.4	96.7	102.1	90.8	86.7	77.9	5.8
0.1M Tris	6.7	91.3	65.6	48.1	14.3	3.3	0	6.4
0.1M NaOAc	6.5	99.9	96.9	98.2	90.7	86.9	75.7	6.3

Data have been obtained concerning the stability of ampicillin in human stool specimens. Sixty-four to eighty-four percent of the ampicillin was recovered after 3-4 week storage of the stool-buffer blend in dry ice. A recovery control consisting of the sample stool-buffer blend dosed with a known amount of ampicillin was prepared for each sample. When a correction is made based on the recovered penicillin from the control, full recovery of ampicillin and stability through 3 weeks is obtained from the stool-buffer mixture⁸². When ampicillin was incubated with the fecal flora of rats and tested for antibiotic activity, only 36.7% of the ampicillin activity was left after 4 hours⁸³.

Gradnik⁸⁴ showed that ampicillin is unstable when injected with penicillinase into rabbits. Synergistic action of ampicillin and cloxacillin has been demonstrated⁸⁵. Cloxacillin inhibits the microbial degradation of ampicillin. The enzymic degradation of ampicillin by extra-cellular and cell-bound penicillinases from an ampicillin resistant strain of Staphylococcus was also inhibited by cloxacillin. The addition of cloxacillin to solutions containing S.aureus penicillinase greatly reduced β -lactam hydrolysis of ampicillin. β -lactam ring hydrolysis with Difco penicillinase is a basis of the spectrophotometric determination of ampicillin⁷⁵. Aminoalkylcatechols have been shown to catalyze the hydrolysis of ampicillin at relatively rapid rates at neutral pH by a mechanism similar to that postulated for several hydrolytic enzymes⁸⁶. Rates of hydrolysis of ampicillin were measured in the presence of several 3,6-bis(aminoalkyl) catechols where the amino group was varied in both size and basicity. Hou and Poole⁸⁷ reported that ampicillin at a pH equal to the isoelectric point exists as

zwitterion and in this form is most stable in aqueous solutions. The stabilization of solutions with acetone has been patented⁸⁸. Kuchinskas and Levy⁸⁹ showed that ampicillin formed polymers in different molecular sizes in aqueous solutions within 1 day that were separable by filtration on columns of an acrylamide gel.

3.3 Stability of Ampicillin Powders

Weiss and Palmer⁹⁰ showed that ampicillin powders were stable when stored in a closed system at 43% and 81% relative humidity at room temperature for 6 weeks. Ampicillin is also stable at 35°C in the same closed system for nine weeks. Ampicillin showed little change in either moisture content or potency.

3.4 Cupric Ion-Catalyzed Hydrolysis

It has been reported^{91,92} that the effect of Cu (II) on the penicillins was to promote their degradation to coordination complexes of Cu (II) and the corresponding penicilloic acids. Complexation was assumed to occur between Cu (II) and the intact penicillins, followed by a rate limiting hydrolysis of the complex into the corresponding penicilloic acid - Cu (II) complex. The reaction mechanism and the catalytic site of complexation of Cu (II) with penicillin have been studied⁹³.

4. Methods of Manufacture

4.1 Microbiological

Ampicillin can be prepared microbiologically by incubation of microbes, e.g. Pseudomonas species, Kluyvera citrophila, Rhodopseudomonas spheroides, or Micrococcus ureae with 6-amino-penicillanic acid and α -phenylglycine⁹⁴, via enzymic acylation of 6-aminopenicillanic acid^{95,96}

or it may be synthesized by the cell-bound penicillin acylase of Escherichia coli⁹⁷. Factors affecting the synthesis of ampicillin by the cell-bound penicillin acylase of Escherichia coli have been studied⁹⁸.

4.2 Chemical

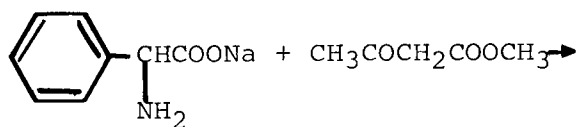
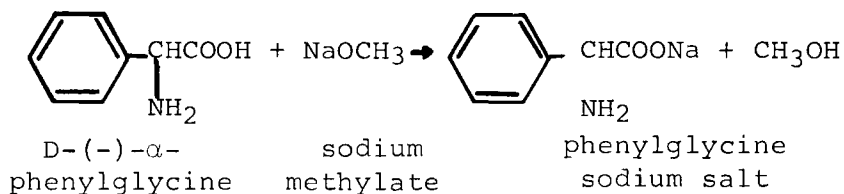
Ampicillin has been synthesized by condensing 6-aminopenicillanic acid (6-APA) with a protected α -phenylglycine. The many groups used to protect the reactive primary amine of α -phenylglycine are summarized in the Table IX. Ampicillin is regenerated by removal of the protective groups. Ampicillin can also be obtained by treating 6-APA with α -bromophenylacetyl bromide¹³¹ via azido penicillin and catalytic hydrogenation^{132,133,134}, the reaction of 6-APA with D(-)-2-phenylglycine chloride¹³⁵ and trimethylsiliconamine and pyridine¹³⁶. Ampicillin is prepared by treating α -bromobenzylpenicillin with hexamethylenetetramine^{137,138} and by Schiff base process¹³⁹. A flowsheet of a plant for the production of synthetic penicillins containing predetermined side chains attached to 6-APA is given¹⁴⁰.

Table IX
Synthesis of Ampicillin

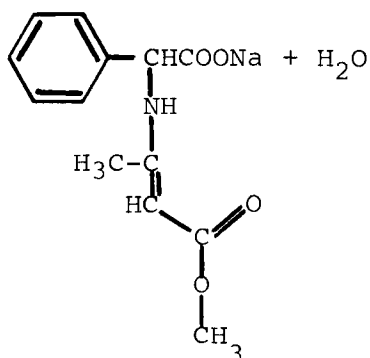
<u>Protective Group on α-Phenylglycine</u>	<u>Reference</u>
2-Nitro-4-methoxyphenylsulfenyl	99
2,5-Oxazolidinedione	100, 101, 102
	103, 104
<u>o</u> -Nitrophenylsulfenyl	110
N-Carboxyanhydride	107, 108, 109
<u>p</u> -Toluenesulfenyl	105, 106
N-(1-Methyl-2-phenylcarbamoylvinyl) anhydride	111
N-Formyl	112
2-Quinol-8-yl-carbonylamino	113
1-3-Oxazine-2,6-dione	114
6-N-benzoyl-2-tyrosyl	115
α -Benzyloxycarbonylamino	116
2,4-Pentanedione (Acetylacetone)	117, 118, 119
N-Carbobenzoxo	120, 121, 122
β -Dicarbonyl (BzCH ₂ Ac, Ac ₂ CH ₂ , AcCH ₂ CO ₂ Et)	123, 124
N(2-Hydroxy-1-naphthylmethylene) anhydride	125
6-2-Phenylcyclohex-3-enyl- carboxamido	126
Furfural, furylacrolein, crotonaldehyde, anisaldehyde and salicylaldehyde	127
Ethoxyformic anhydride	128
Phenylsulfide (PhSH)	129
<u>o</u> -Acetoacetanisidine	130

AMPICILLIN

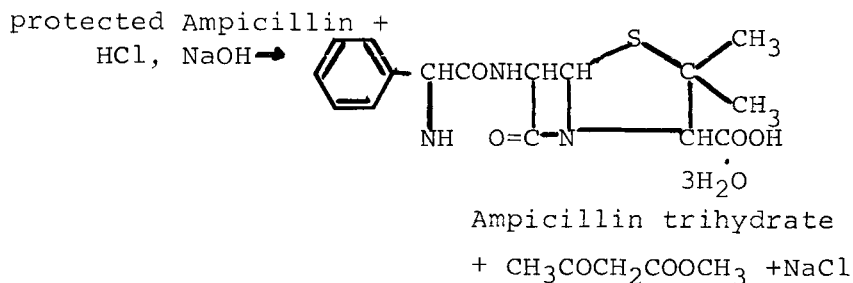
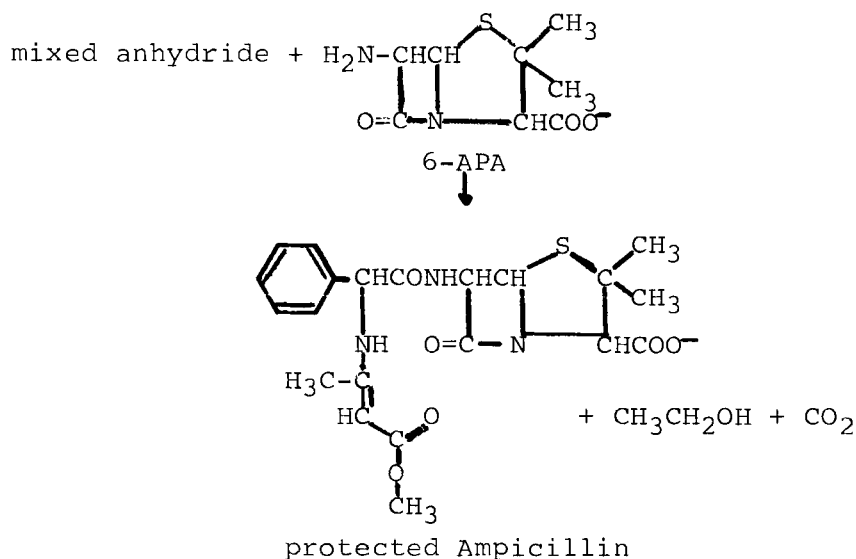
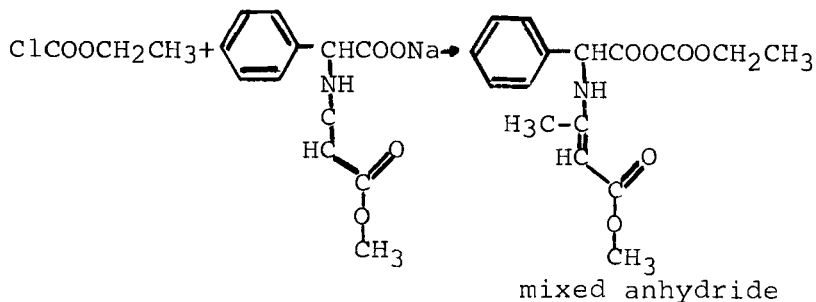
Production of ampicillin via methyl N(α -carboxy-benzyl)- β -aminocrotonate is illustrated. The process for the preparation of the protected α -phenylglycine has been patented^{141,142} and can be summarized as follows:



methyl acetoacetate



The patented process for the synthesis of ampicillin trihydrate¹¹⁷⁻¹¹⁹ can be outlined:



5. Isolation and Purification

Ampicillin can be purified by acid or alkaline recrystallizations. It is dissolved in a mineral acid or caustic solution and precipitated by the adjustment of pH⁶⁴. Crude ampicillin is purified by dissolution in methylene dichloride containing triethylamine, filtration and precipitation with p-toluene sulfonic acid¹⁴³. Ampicillin is separated from 6-aminopenicillanic acid by the filtration of chloroform or methylene chloride containing triethylamine and evaporation of the solvent¹⁴⁴. Antigenic impurities are removed from solution by gel filtration¹⁴⁵⁻¹⁴⁸. A process is described for the isolation of ampicillin via adduct formation with aliphatic amines and precipitation with 6-amino-1,3-naphthalene-disulfonic acid¹⁴⁹.

Ampicillin was separated from dicloxacillin and cloxacillin on an ion exchange column, IRA-402 resin¹⁵⁰. Ampicillin was extracted with ion-pairing and adduct-forming agents¹⁵¹. Purification of ampicillin via Schiff's bases, sulfonic acid salts, amine salts and Tergitol salts has been evaluated¹⁵².

6. Methods of Analysis

6.1 Identification Tests

Ampicillin was identified by infrared spectroscopy¹⁵³. Chromotropic acid, sulfuric acid, ninhydrin and potassium cupri-tartrate tests² are used to identify ampicillin. Electrophoresis in an agar gel is used for separation of penicillins. Spots are detected microbiologically¹⁵⁴. Thomas and Broadbridge¹⁵⁵ described a method for the rapid separation and detection of mixtures of penicillins by low voltage electrophoresis. The penicillins are

identified microbiologically.

Sephadex thin layer chromatography and bioautography were combined for the identification of antibiotics¹⁵⁶. Identity tests for antibiotics sensitivity discs were developed¹⁵⁷. Several thin layer chromatography methods for identification of penicillin are reported¹⁵⁸⁻¹⁶². A simple and rapid polyamide chromatography of penicillins is described¹⁶³. (See Section 6.2.4 for additional systems).

Paper chromatography is used for the separation and identification of different penicillins¹⁶⁴⁻¹⁶⁸. The R_f values of separated samples are compared with those of standards. (See section 6.2.5 for additional methods).

6.2 Quantitative Methods

6.21 Ultraviolet Spectrophotometric Methods

A spectrophotometric method for the determination of ampicillin involving initial benzylation of the side chain α -amino group is described. α -Benzamidobenzylpenicillin so formed is treated with mercuric chloride in acid solution and α -benzamidobenzylpenicillenic acid is obtained. This may be assayed spectrophotometrically¹⁶⁹. Ampicillin is degraded in a buffer solution at pH 5.2 at 75°C and the absorbance is measured at 320 nm¹⁷⁰. Smith's method for the spectrophotometric determination of ampicillin was adapted to the assay of ampicillin in chicken blood, bile and urine¹⁷¹. Ampicillin is dissolved in 5N sodium hydroxide and the absorbance is measured at 279 nm¹⁷². Ampicillin is determined spectrophotometrically as its copper complex¹⁷³. Spectrophotometric and circular dichroism methods for determining the activity of ampicillin are described¹⁷⁴. A method based on the degradation of the β -lactam ring with penicillinase and formation of a

copper chelate is given¹⁷⁵.

6.22 Fluorometric Determination

A method for the fluorometric analysis of ampicillin in biological fluids is described¹⁷⁶. Ampicillin forms a strongly fluorescent yellow product in acid solution at elevated temperatures. A fluorescent thin layer chromatography procedure for determining trace ampicillin involves the reaction of hydrolyzed penicillin with a fluorescent isothiocyanate to form the corresponding fluorescent thiourea¹⁷⁷.

6.23 Polarographic Determination

Ampicillin can be determined at p.p.m. levels by differential pulse polarography¹⁷⁸. Ampicillin undergoes reduction at a mercury electrode and the peaks obtained can be used for both quantitative and qualitative analyses. Peak heights are linear with concentration.

6.24 Thin Layer Chromatography

A thin layer chromatographic assay of ampicillin and cloxacillin in body fluids was developed. Penicillins are separated on silica gel. Spots were obtained using a bioautographic technique¹⁷⁷. Mixtures of synthetic penicillins are separated on a silica gel sheet and developed with butanol:ethanol:water (4:1:5)¹⁸⁰ and zones of inhibition were obtained with Bacillus subtilis ATCC6633. Mixtures of ampicillin and oxacillin were separated by thin layer chromatography on silica gel and the antibacterial activity was measured against Sarcina lutea¹⁸¹. A thin layer chromatographic method for semisynthetic penicillins and tetracyclines is described¹⁸². Antibiotics are separated on silica gel plates using water or pH 6.1 McIlvaine's buffer solution

as solvent. Biagi et al.¹⁸³ used reversed phase thin layer chromatography on silica gel impregnated with silicone DC200 to develop R_f values for various penicillins. Reversed-phase thin layer chromatography was used to examine the effect of the pH of a mobile phase on the partitioning of various penicillins between a polar mobile phase and nonpolar stationary phase¹⁸⁴. A quantitative thin layer chromatographic procedure for measuring ampicillin is described. Silica gel plates impregnated with silicone fluid are used. McIlvane's pH 4.1 buffer containing 1.5% acetone is employed for development. The spots are visualized with ninhydrin. A color is eluted and measured spectrophotometrically¹⁸⁵.

6.25 Paper Chromatography

A paper chromatographic technique for determination of ampicillin in epicillin samples has been developed¹⁸⁶. Paper chromatograms were developed with a water saturated mixture of n-butanol and t-amyl alcohol (6:1) vs pH 4.2 McIlvane's buffer. Penicillins were detected biologically¹⁸⁶. Roberts and Vahidi¹⁸⁷ described a paper chromatographic procedure for the determination of ampicillin. Papergrams are developed in n-butanol, t-amyl alcohol and water (7:1:4) for 48 hours. Bioautograms of the strips are prepared using S. aureus plates. Paper chromatography of ampicillin samples was carried out on Whatman No. 1 paper¹⁸⁸. The following systems were used: butanol, ethanol, water (4:1:5), butanol, acetic acid, water (12:3:5), butanol, pyridine, water (1:1:1), methanol, pyridine, concentrated hydrochloric acid, water (160:20:4:35), ammonia, propanol, water (3:6:1). After descending chromatography papers were sprayed with ninhydrin or developed bioautographically

using Staphylococcus aureus¹⁸⁸. Two dimensional paper chromatography for the determination of ampicillin in urine was used¹⁸⁹. Uri¹⁹⁰ inoculated agar plates in parallel stripes with 16 microbial cultures of gram-positive, gram-negative and acid resistant microorganisms and with Candida albicans. The growth zones are then partially covered with a filter paper strip containing the separated antibiotics to be tested. The zones of inhibition were measured and concentration of ampicillin calculated.

6.26 Iodometric Titration

Alicino¹⁹¹ described an iodometric method for the determination of penicillins. Only after alkaline or penicillinase hydrolysis the penicillins consume iodine. The difference in consumption of iodine before and after hydrolysis is proportional to the quantity of the antibiotic. Russo-Alesi¹⁹² used the iodometric titration for estimation of ampicillin in formulations. Ampicillin solutions were hydrolyzed with sodium hydroxide for 30 minutes. After acidification the iodine solution was added. After an additional 30 minutes, the excess of iodine was titrated with thiosulfate solution. A blank consisted of unhydrolyzed ampicillin. The ampicillin standard was treated in exactly the same manner and used in calculations. The hydrolysis of ampicillin with penicillinase instead with sodium hydroxide makes the method more selective.

An automated system for the iodometric determination of penicillins using an AutoAnalyzer is described¹⁹³. The accuracy of the method is from 1 to 3% and the precision is 2 to 3.4%.

6.27 Hydroxamic Acid

The formation of a colored chelate of ferric ion and the hydroxamic acid of benzyl-

penicillin is the basis of the colorimetric method¹⁹⁴. The color produced can be extracted into butanol¹⁹⁵. Details of the procedure was published¹⁹⁶.

The automated method¹⁹⁷ for the determination of benzylpenicillin can be used for the determination of ampicillin. Penicillins react with hydroxylamine and ferric ion to produce a color which is measured continuously with an AutoAnalyzer.

6.28 Amperometric Titration

Ampicillin is hydrolyzed to penicillamine. The SH group of penicillamine is titrated amperometrically. The author claims that the method determines only the biologically active penicillins and not the degradation products¹⁹⁸.

6.29 Microbiological Methods

A complete description is given for the laboratory equipment, solutions, culture diffusion and turbidimetric assays used as tests and methods for the assay of ampicillin¹⁹⁹. Yakovlev²⁰⁰ determined ampicillin in blood serum by an agar-diffusion technique. Staphylococcus aureus 209P and Bacillus subtilis were used as test organisms. A modified AutoAnalyzer method of Shaw and Duncombe²⁰¹ is used for the determination of ampicillin²⁰². The inoculum was Escherichia coli 397E grown on tryptone soya agar, prepared by the method of Dewart²⁰³. Grimshaw and Jones²⁰⁴ presented an automated method for the determination of ampicillin. The method produces a linear response for ampicillin over an 80-180 mcg/ml range. An automated turbidimetric system for ampicillin assay is described²⁰⁵.

7. Protein Binding

Quinn, et al.,²⁰⁶ found that ampicillin was less highly bound to serum than either benzylpenicillin or phenoxymethylpenicillin. Serum had little effect on the activity of ampicillin²⁰⁷. When the Oxford Staphylococcus and a strain of Escherichia coli were used as test organisms, the minimal inhibitory concentrations for ampicillin in the presence of 40% human serum were only one tube lower than in the controls. The binding of ampicillin to proteins was investigated²⁰⁸. Phenol red proved to be the most suitable for comparison of the binding of penicillins to serum proteins. Investigations with albumin treated with dinitrofluorobenzene proved that primary reactive amino groups of the albumin molecules are responsible for the binding of penicillins. Binding of penicillins, including ampicillin, with serum proteins were studied, too²⁰⁹. Penicillins were not bound by human globulin but a large amounts, except ampicillin, was bound with 1% albumin. Lipid solubility and serum protein binding of various penicillins were correlated²¹⁰. Robinson and Sutherland²¹¹ studied the binding of penicillins and other antibiotics in serum by ultrafiltration technique. New data were given on the binding of penicillins to the proteins in human serum²¹². The extent of binding was determined by dialysis, plate tests and gel filtration. A new approach to studying the protein binding of penicillins has been reported²¹³. Gel-filtration and both static and continuous dialysis methods were used to examine protein binding of penicillins. Results showed that penicillins differ in the degree of binding. The relationship between the extent of serum-protein binding of penicillins and the corresponding effluent volume from Sephadex G-25, and the relationship between the protein-binding

extent and the gel-affinity is described²¹⁴. Penicillins showing an increase in serum binding also showed an increase in the effluent volume. Estimation and use of serum levels in the evaluation of the new penicillin is presented²¹⁵. The degree of binding was established by filtration under negative pressure through a dialysis membrane. The binding rates of penicillins to mixtures of albumin, euglobulin, and pseudoglobulin fraction were nearly the same as those of the whole bovine serum²¹⁶. The binding rate of the mixture of any 2 or 3 of the serum protein fractions is always lower than the sum of those to corresponding individual fraction. Binding rates and the modes of binding of penicillins with various proteins in vivo and in vitro were investigated by ultrafiltration and dialysis²¹⁷. Kunin²¹⁸ reported that only 23% of ampicillin was bound to human serum. Serum proteins bind ampicillin only loosely²¹⁹. Ampicillin was hardly absorbed on human red cells, slightly inactivated with mouse liver homogenates and was combined slightly with horse serum proteins²²⁰. The effect of serum binding on the distribution of penicillins in the rabbit has been studied²²¹. ¹⁴C-labeled penicillins were used and their distribution in brain, muscle, lung and heart was measured and correlated to binding. The role of ampicillin in antibiotic therapy and its serum binding is given²²². Differences in serum binding among penicillins exert a profound effect upon the amount of free drug available for antimicrobial activity²²³. Kunin²²⁴ found that ampicillin showed less binding to serum proteins than did dicloxacillin, cloxacillin, oxacillin, nafcillin, penicillins G and V and methicillin. An antimicrobial activity of penicillins is increased by competitive serum binding inhibitors²²⁵. Immunochemical studies on the antigenic bindings

of ampicillin and other penicillins were conducted²²⁶. The amount of ampicillin bound to the protein was established by dialysis and with p-chloromercuribenzoate titration of the penicilloyl groups and by the formol titration of protein amino groups²²⁷.

8. Pharmacokinetics

Pharmacokinetics of ampicillin trihydrate and sodium ampicillin following intramuscular injection were studied ²²⁸. Serum levels of ampicillin activity were determined. The rates of absorption of the drug from the intramuscular injection sites were calculated. The ampicillin suspension data showed that absorption process is slower than the elimination process. Ampicillin sodium solutions are absorbed more efficiently than ampicillin trihydrate. Klein et al.²²⁹ gave three intramuscular injections of ampicillin to eight apparently healthy young men. Doses of 1.0 g., and 0.5 g. were given at two-day intervals. Absorption and excretion of intramuscular doses of ampicillin appeared to be somewhat delayed when compared with penicillins G and V. Peak serum levels of ampicillin appeared later and significant antibacterial activity was also demonstrated. On the average, about twice as much ampicillin was recovered in the urine after intramuscular administration than when same amounts had been given orally. Bunn²³⁰ found that peak serum concentration after a 500 mg intramuscular dose of ampicillin was attained within 20-30 minutes. Urinary excretion amounted to 40% of the dose within the first 8 hours. Bunn, et al. found also that peak serum concentration of ampicillin was attained within one hour after intramuscular injection of 500 mg, 750 mg or 1000 mg of ampicillin²³¹. Probenecid increases and sustains the levels of ampicillin in the serum after intra-

muscular injection of the antibiotic²³². The excretion of ampicillin into the urine is delayed, prolonged and amount reduced. The combination of injected ampicillin and oral probenecid has been used^{233,234,235}. When ampicillin is given orally in doses of 250 mg, 500 mg, 750 mg, and 1000 mg peak serum concentrations are obtained at about 2 hours after dosing²³⁶. Significant serum concentrations of ampicillin are present at six hours of the dosing. Doubling the dose doubles the peak serum concentration. About 30% of a given dose is excreted in the urine 6-8 hours. Ampicillin concentration is not affected by food²³⁷ and antibacterial activity of serum samples is proportional to dosage.

Bunn²³⁸ found that 25% of an oral dose of ampicillin is excreted in the first 8 hours, and that the blood levels of ampicillin at 4 hours were the same after doses of 250, 500, or 1000 mg. Stewart et al.²³⁹ showed that inhibitory levels of ampicillin are attained and maintained between one and one-half and seven hours after oral doses of 100 mg/kg/day. With lower doses inhibitory concentrations are maintained for one and one-half to five hours. About 30-50% of the total dose given over a period of five to seven days was excreted. Chromatographic bioassays of the excretion product in the urine suggested that it was largely ampicillin and not a metabolite.

9. References

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CHLORPROTHIXENE

Bruce C. Rudy and Bernard Z. Senkowski

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Analytical Profile - Chlorprothixene

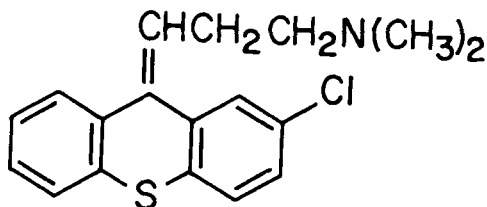
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CHLORPROTHIXENE

1. Description

1.1 Name, Formula, Molecular Weight

Chlorprothixene is 2-chloro-N,N-dimethyl-thioxanthene- Δ^9 , γ -propylamine.



C₁₈H₁₈ClNS

Molecular Weight: 315.87

1.2 Appearance, Color, Odor

Chlorprothixene is a light yellow to yellow crystalline powder with a light amine-like odor.

1.3 Isomeric Forms

Chlorprothixene exists as a cis- or a trans- isomer. The cis- isomer has also been referred to as the α -isomer or the high melting isomer (1,2). The trans- isomer likewise has been referred to as the β -isomer or low melting isomer. This work will deal primarily with the α -isomer.

2. Physical Properties

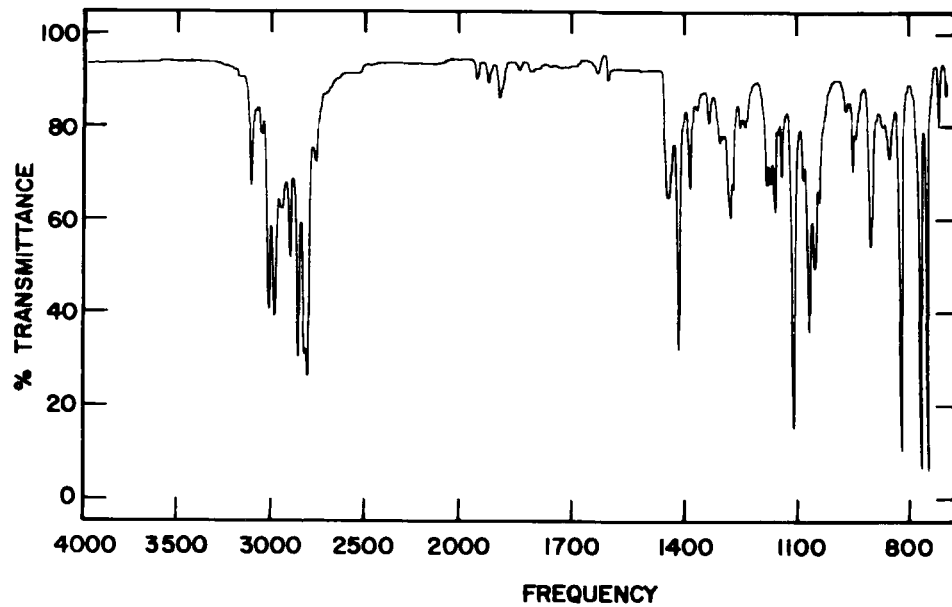
2.1 Infrared Spectrum

The infrared spectrum of bulk reference standard chlorprothixene is shown in Figure 1 (3). The spectrum of a 10% carbon disulfide solution of chlorprothixene (w/v) was measured versus carbon disulfide in 0.1 mm NaCl liquid cells on a Perkin Elmer 621 Spectrophotometer.

The following assignments have been given to the bands in Figure 1 (3):

- Characteristic for aromatic CH: 3063 cm⁻¹
- Characteristic for CH₂ stretching: 2939 and 2854 cm⁻¹
- Characteristic for CH₃ stretching: 2816 and 2767 cm⁻¹

Figure 1
Infrared Spectrum of Chlorprothixene



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- d. Characteristic for 4 free H on benzene ring:
757 to 739 cm^{-1}
- e. Characteristic for 2 free H on benzene ring:
809 cm^{-1}

2.2 Nuclear Magnetic Resonance Spectrum (NMR)

The NMR spectrum shown in Figure 2 was obtained by dissolving 52.2 mg of reference standard chlorprothixene in 0.5 ml of CDCl_3 containing tetramethylsilane as the internal reference. The spectral assignments are shown in Table I (4).

TABLE I

NMR Spectral Assignments for Chlorprothixene

Type Protons	No. of Each Proton	Chemical Shift (ppm)	Multiplicity
methyl	6	2.24	s
methylene	4	2.35-2.80	m(u)
vinyl	1	5.97	t(u)
aromatic	7	7.10-7.60	m(u)

s = singlet; m(u) = unsymmetrical multiplet; t(u) = unsymmetrical triplet

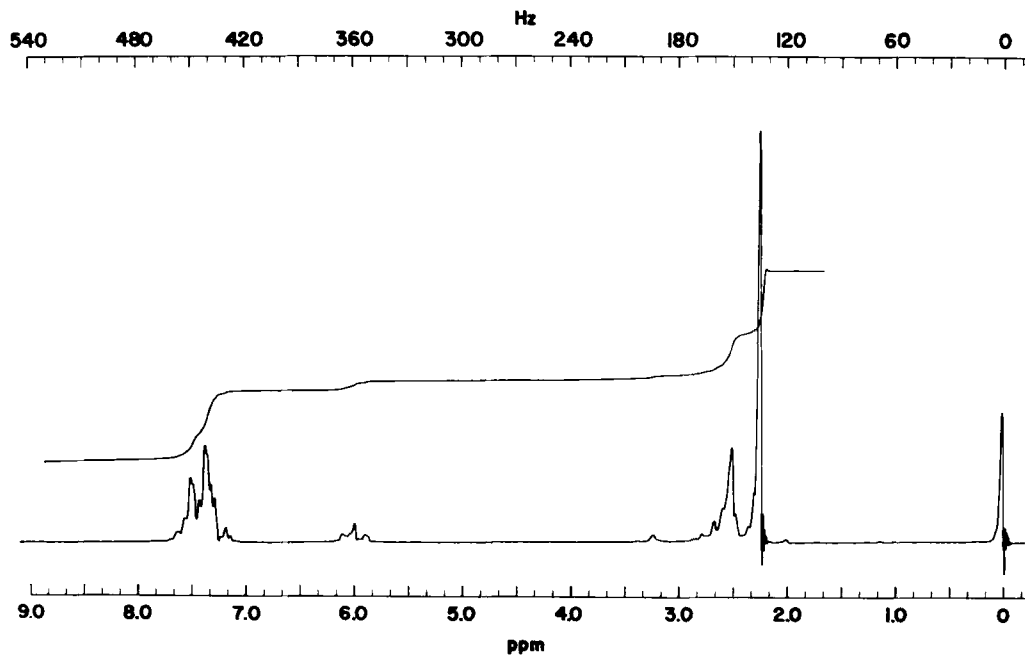
2.3 Ultraviolet Spectrum

The ultraviolet spectrum of chlorprothixene in 0.1N HCl in the region 400 to 210 nm exhibits three maxima and three minima. The maxima are located at 229 - 230 nm ($\epsilon = 3.4 \times 10^4$), 267 - 268 nm ($\epsilon = 1.3 \times 10^4$), and 323 - 324 nm ($\epsilon = 2.8 \times 10^3$). The minima were observed at 217 nm, 254 nm, and 307 - 308 nm. The spectrum presented in Figure 3 was obtained from a reference standard solution of chlorprothixene at a concentration of 0.602 mg per 100 ml of 0.1N HCl (3).

2.4 Fluorescence Spectrum

Figure 4 shows the excitation and emission spectra for reference standard chlorprothixene from 260 to 540 nm (5). The spectra were measured on a 0.1N HCl solution of chlorprothixene (0.5 mg/ml) using a Farand MK-1 spectro-fluorometer. Excitation at either 310 nm or 352 nm produced

Figure 2
NMR Spectrum of Chlorprothixene



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Figure 3
Ultraviolet Spectrum of Chlorprothixene

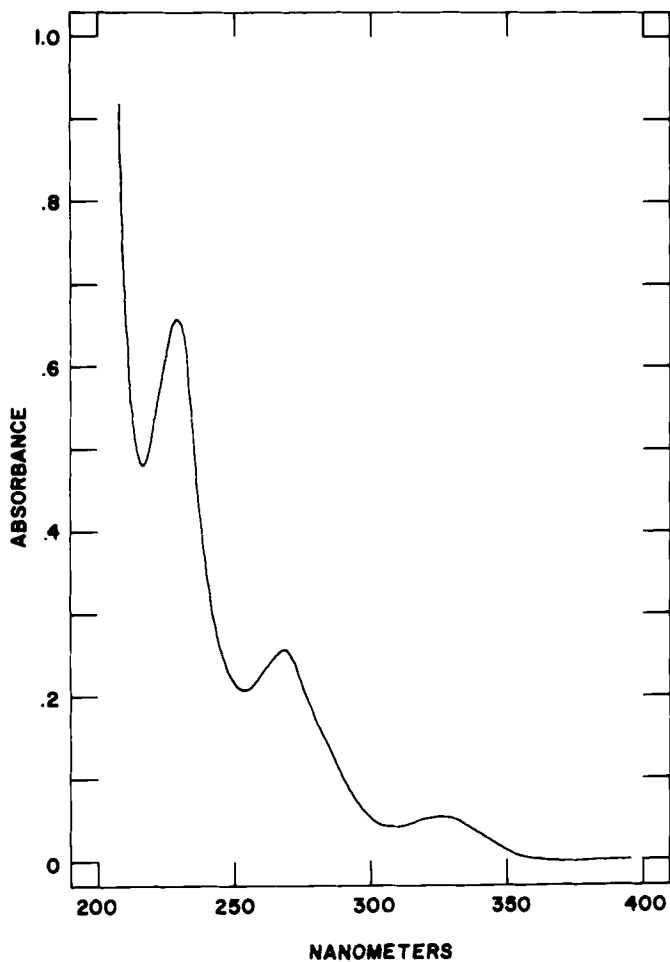
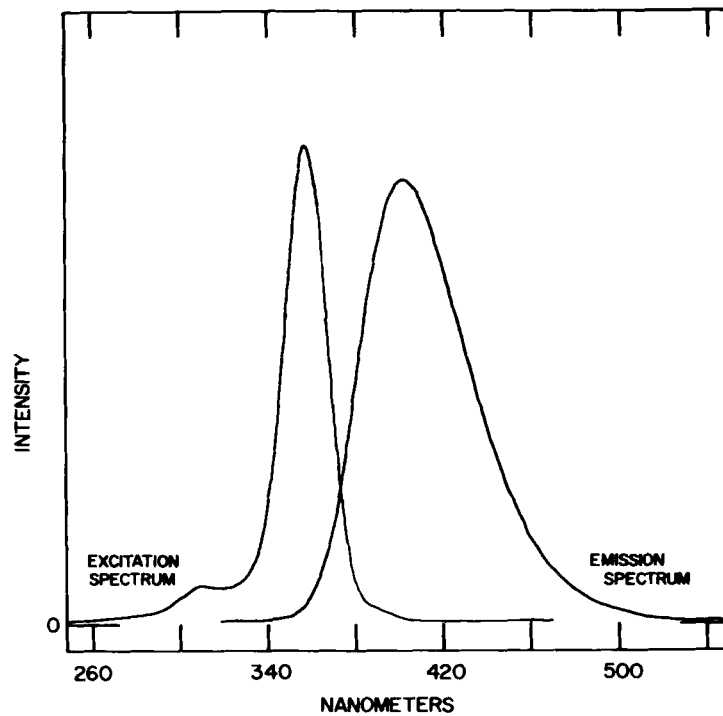


Figure 4
Fluorescence Spectra of Chlorprothixene



identical emission spectra with a maximum at 401 nm.

2.5 Mass Spectrum

The mass spectrum of chlorprothixene reference standard was obtained using a CEC 21-110 spectrometer with an ionizing energy of 70 eV (Figure 5) (6). The low resolution spectrum showed the following diagnostic peaks:

<u>mass (m/e)</u>	<u>Intensity</u>
315	very weak
313	very weak
271	very weak
257	weak
255	medium-weak
234	weak
221	strong
189	medium-weak
58	very strong

The molecular ion at m/e 315 and the fragment ions at m/e 271, 257, and 255 display the expected chlorine isotope peak 2 mass units higher. The base peak at m/e 58 and the ion at m/e 257 are both due to cleavage beta to the amine function. The peak at m/e 255 could be explained as loss of two hydrogens from m/e 257 possibly leading to a ring-closed structure. The peak at m/e 221 is due to the loss of HCl from m/e 257. Since the spectra did not show any change with time it is likely that m/e 313 is a fragment ion derived from m/e 315. Again, a ring closure type of structure is a possibility (6).

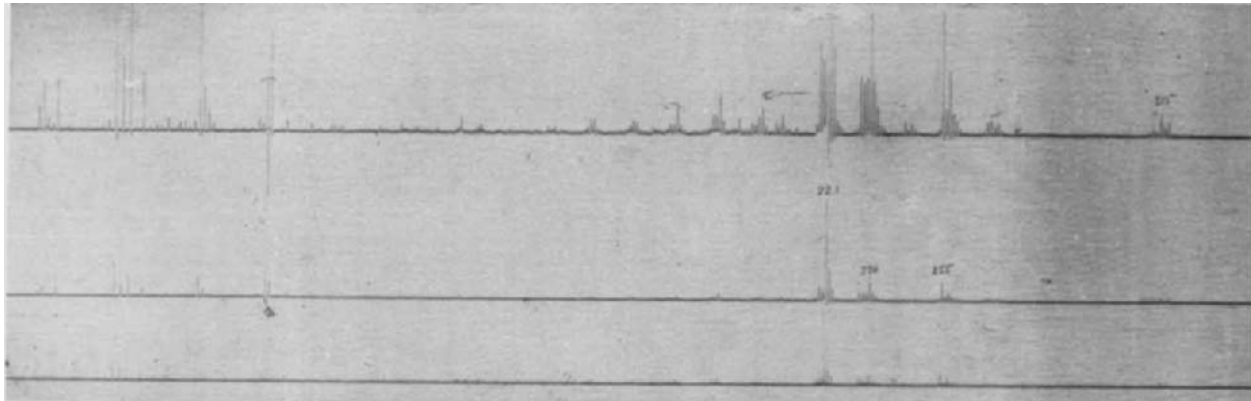
2.6 Optical Rotation

Chlorprothixene exhibits no optical activity.

2.7 Melting Range

A sharp melting point is not observed with chlorprothixene. The melting depends on the rate of heating. The melting range reported in NF XIII is 96.5° to 101.5°C.

Figure 5
Mass Spectrum of Chlorprothixene



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2.8 Differential Scanning Calorimetry (DSC)

The DSC curve for reference standard chlorprothixene was obtained using a Perkin Elmer DSC - 1B Calorimeter. With a temperature program of 10°C/min., a melting endotherm was observed starting at 92.8°C (shown in Figure 6) and another endotherm starting at 246°C which corresponds to the decomposition of the chlorprothixene. The ΔH_f was found to be 7.4 kcal/mole for the melting endotherm (7).

2.9 Thermogravimetric Analysis (TGA)

The TGA performed on reference standard chlorprothixene exhibited no loss of weight when heated to 105°C at a heating rate of 10°C/min (7).

2.10 Solubility

The solubility data obtained at 25°C for reference standard chlorprothixene is listed in Table II (8).

TABLE II

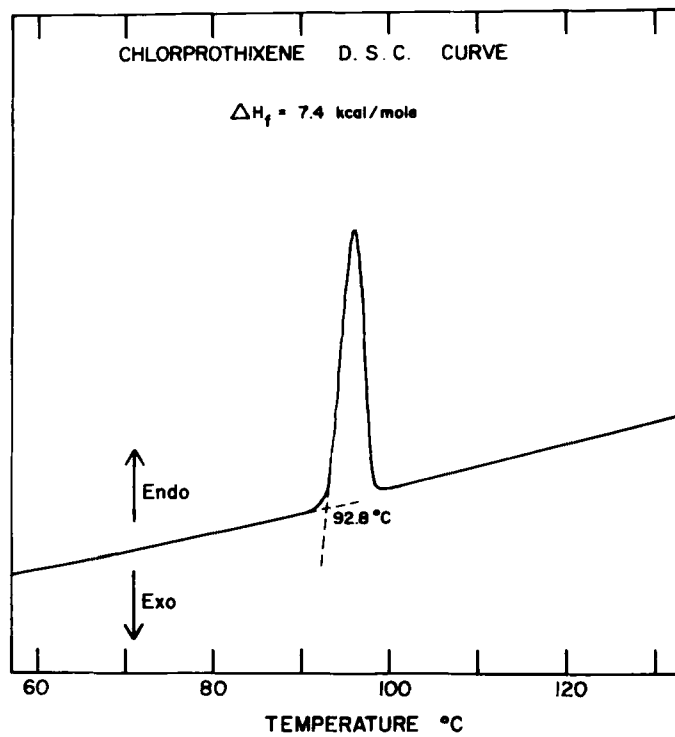
Chlorprothixene - Solubility

<u>Solvent</u>	<u>Solubility (mg/ml)</u>
3A alcohol	36.7
benzene	30.5
chloroform	>500
95% ethanol	28.2
ethyl ether	77.6
isopropanol	22.0
methanol	36.5
petroleum ether (30°-60°)	16.9
water	<0.1

2.11 Crystal Properties

The x-ray powder diffraction pattern of reference standard chlorprothixene is presented in Table III (9). The instrumental conditions are given below:

Figure 6



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Instrumental Conditions:

General Electric Model XRD-6 Spectrogoniometer

Generator: 50 KV, 12-1/2 MA

Tube Target: Copper

Radiation: Cu K α = 1.542 Å

Optics: 0.1° Detector slit

M.R. Soller slit

3° Beam slit

0.0007" Ni filter

4° take off angle

Goniometer: Scan at 0.2° 2 θ per minute

Detector: Amplifier gain - 16 coarse,

8.7 fine

Sealed proportional counter tube

and DC voltage at plateau

Pulse height selection E_L- 5 volts

E_u-out

Rate meter T.C. 4

2000 C/S full scale

Recorder: Chart Speed 1 inch per 5 minutes

Samples: Prepared by grinding at room temperature

2.12 Dissociation Constant

The apparent pK_a for chlorprothixene has been determined spectrophotometrically to be 8.4 (10). The apparent pK_a has also been determined from the titration curve in an isopropanol:water (1:1) mixture and found to be 7.5 (11). In water, the trialkylamino type compounds are stronger bases, on the average, by 0.9 pK units (11,12). Therefore, the estimated pK_a in water is 8.4 which is in good agreement with that found spectrophotometrically.

TABLE III

X-ray Diffraction Pattern of Chlorprothixene

2θ	$d^* \text{ \AA}$	I/I_0^{**}
8.18	10.81	100
14.49	6.11	21
16.39	5.41	62
16.67	5.32	45
19.55	4.54	75
21.43	4.15	24
22.71	3.92	8
23.00	3.87	10
24.24	3.67	31
24.55	3.63	29
25.86	3.45	15
26.33	3.38	3
26.82	3.32	28
27.53	3.24	6
28.69	3.11	10
29.69	3.01	3
30.47	2.93	15
31.37	2.85	1
33.12	2.70	8
33.48	2.68	1
33.79	2.65	2
34.40	2.61	3
35.03	2.56	3
35.92	2.50	4
37.25	2.41	2
37.81	2.38	3
38.18	2.36	1
39.00	2.31	1
39.65	2.27	1
40.80	2.21	3
41.80	2.16	17
42.62	2.12	2
43.23	2.09	3
44.36	2.04	3
45.06	2.01	4
45.70	1.99	2
49.55	1.84	2
50.68	1.80	5

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TABLE III (cont.)

X-ray Diffraction Pattern of Chlorprothixene

2θ	$d^* \text{ \AA}$	I/I_0^{**}
52.30	1.75	2
54.10	1.70	2

$$*d - (\text{interplanar distance}) \quad \frac{n\lambda}{2 \sin \theta}$$

** I/I_0 = relative intensity (based on highest intensity of 1.00)

3. Synthesis

Chlorprothixene may be prepared by the reaction scheme shown in Figure 7. 2-Chlorothioxanthone is reacted with dimethylaminopropyl chloride in the presence of magnesium to form 2-chlorothioxanthanol which is then dehydrated to give chlorprothixene (13).

4. Stability Degradation

Chlorprothixene has been shown to be stable when heated for one hour at 100°C in 0.1N HCl, 0.1N NaOH, and water. (14) When it is subjected to ultraviolet radiation or strongly basic conditions, 2 chlorothioxanthene and 2-chlorothioxanthone are formed which can be detected by TLC or paper chromatography. Also the odor of volatile amines is apparent (15).

5. Drug Metabolic Products

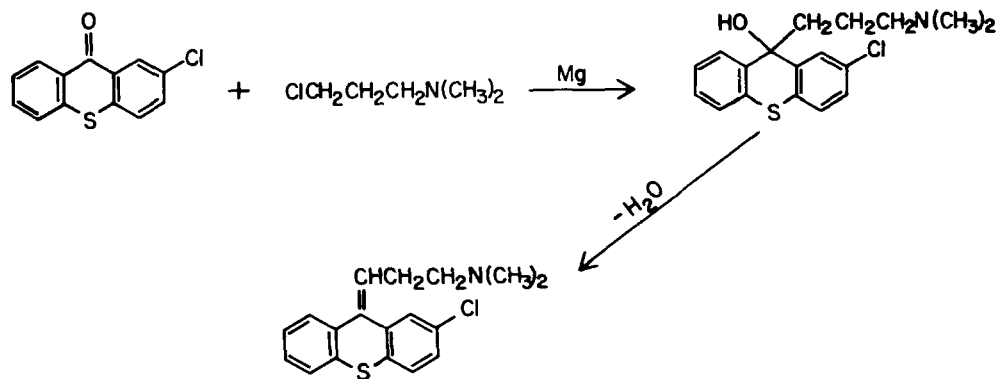
Chlorprothixene is metabolized to the sulfoxide which can be either N-demethylated or further oxidized to the N-oxide, Figure 8 (2,15). The analytical procedures for the metabolites have been described by de Silva (17).

6. Methods of Analysis

6.1 Elemental Analysis

The results from an elemental analysis of reference standard chlorprothixene is presented in Table IV (18).

Figure 7
Synthesis of Chlorprothixene



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Figure 8

Metabolic Products of Chlorprothixene (2)

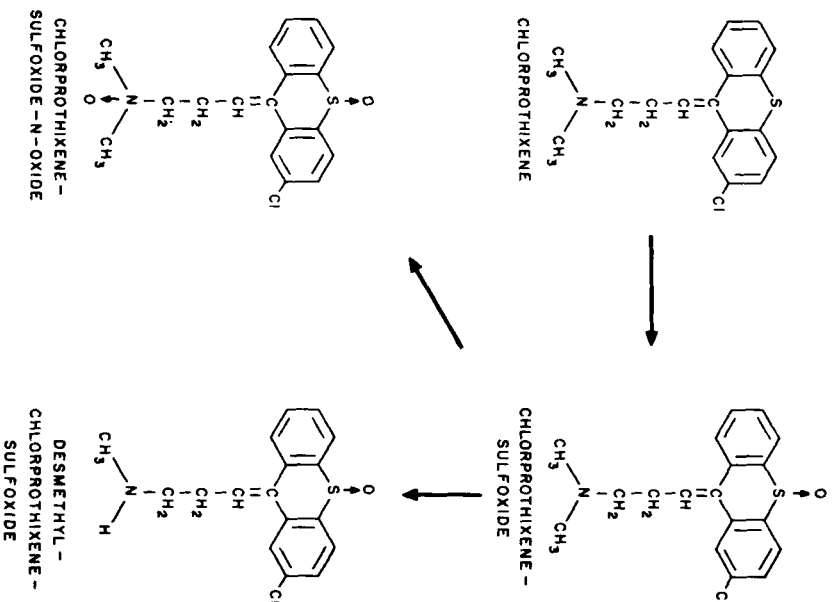


TABLE IV

Elemental Analysis of Chlorprothixene

<u>Element</u>	<u>% Theory</u>	<u>% Found</u>
C	68.45	68.36
H	5.74	5.72
N	4.43	4.45
S	10.15	10.26
Cl	11.22	11.36

6.2 Phase Solubility Analysis

Phase solubility analysis has been carried out for chlorprothixene using either isopropanol or acetonitrile as the solvent. An example is presented in Figure 9 for the reference standard chlorprothixene along with the conditions under which the analysis was carried out (8).

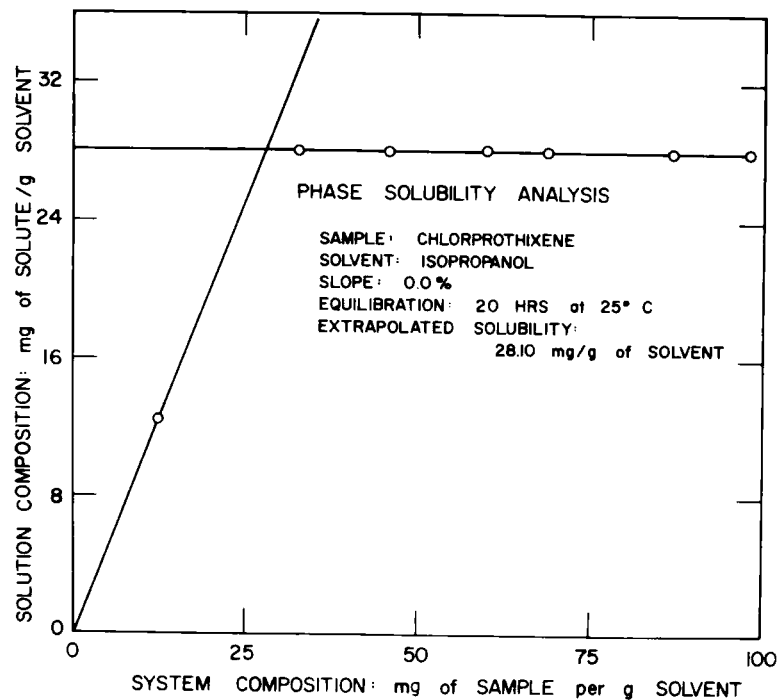
6.3 Thin Layer Chromatographic Analysis (TLC)

The following TLC procedure is useful for separating chlorothioxanthone and chlorothioxanthene from α -chlorprothixene (19). Using silica gel G plates and benzene:heptane (1:1) as the solvent system, 0.1 mg of the sample in anhydrous 3A alcohol is spotted on the plate and subjected to ascending chromatography. After the solvent front has ascended 10 to 15 cm, the plate is air dried and sprayed with concentrated sulfuric acid. The plate is then viewed under shortwave ultraviolet radiation. The approximate R_f values are as follows:

α -chlorprothixene	0.0	(orange fluorescence)
chlorothioxanthone	0.35	(greenish fluorescence)
chlorothioxanthene	0.85	(orange fluorescence)

The TLC procedure found useful for the separation of β -chlorprothixene and 2-chlorothioxanthanol is described below (19). A silica gel G plate is spotted with 0.1 mg of sample and developed 10 to 15 cm using absolute ethanol:benzene:water (20:20:1) as the solvent system. The plate is air dried, sprayed with concentrated sulfuric acid, and viewed under shortwave ultraviolet radiation. The approximate R_f values are as follows:

Figure 9



α -chlorprothixene	0.65 (orange fluorescence)
β -chlorprothixene	0.50 (orange fluorescence)
2-chlorothioxanthenol	0.35 (reddish fluorescence)

6.4 Direct Spectrophotometric Analysis

The chlorprothixene content in tablets may be determined spectrophotometrically by using the following procedure. The tablets are finely ground, a portion of the powder is weighed, and then dispersed by shaking in a 1% ammonium hydroxide solution. The chlorprothixene is extracted with ethyl ether. The chlorprothixene is extracted from the ethereal solution with 0.5N HCl and diluted to volume with 0.5N HCl. Suitable aliquots are further diluted to give a solution containing about 50 $\mu\text{g/ml}$ and the absorbance measured at 324 nm. The concentration of chlorprothixene is calculated using the absorbance obtained from a solution of chlorprothixene reference standard similarly prepared and measured (5,20).

The system used with the Technicon Autoanalyzer for measuring the content uniformity of individual chlorprothixene tablets is as follows. Each tablet is crushed and allowed to stand in 5 ml of dimethylformamide for about 20 minutes. This solution is diluted with 0.1N H_2SO_4 until the concentration is about 50 $\mu\text{g/ml}$. The absorbance is measured at 324 nm and the amount of chlorprothixene calculated by comparison to a solution of reference standard chlorprothixene similarly prepared and measured.

6.5 Colorimetric Analysis

Colorimetric analysis may be accomplished by dissolving the chlorprothixene in concentrated sulfuric acid and diluting an aliquot with 50% sulfuric acid to a final concentration of approximately 0.04 mg/ml. The orange color is measured at 490 nm and the concentration obtained by means of a calibration curve (15).

6.6 Fluorescence Analysis

Fluorescence analysis has proven to be a valuable method for the analysis of chlorprothixene in biological samples. A sensitivity limit of 0.010 micrograms/ml of blood has been reported when the blood is extracted and the extract dissolved in cold concentrated sulfuric acid (17).

6.7 Non-Aqueous Titration

The non-aqueous titration as described in the NF XIII is the method of choice for the analysis of bulk chlorprothixene (20). The sample is dissolved in chloroform, ethanolic methyl red indicator added, and the titration carried out with HClO_4 in dioxane. Each ml of 0.1N HClO_4 is equivalent to 31.59 mg of chlorprothixene.

7. Acknowledgements

The authors wish to acknowledge the Scientific Literature Department of Hoffmann-La Roche Inc. and Dr. A. MacDonald for their assistance in the preparation of this analytical profile.

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CHLORAL HYDRATE

John E. Fairbrother

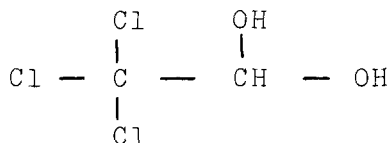
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1. Description1.1 Name, Formula, Molecular Weight

Chloral Hydrate (Chloralis Hydras; Chloratum Hydratum) also known as 2,2,2-Trichloroethane-1,1-diol and trichloroacetaldehyde mono-hydrate.

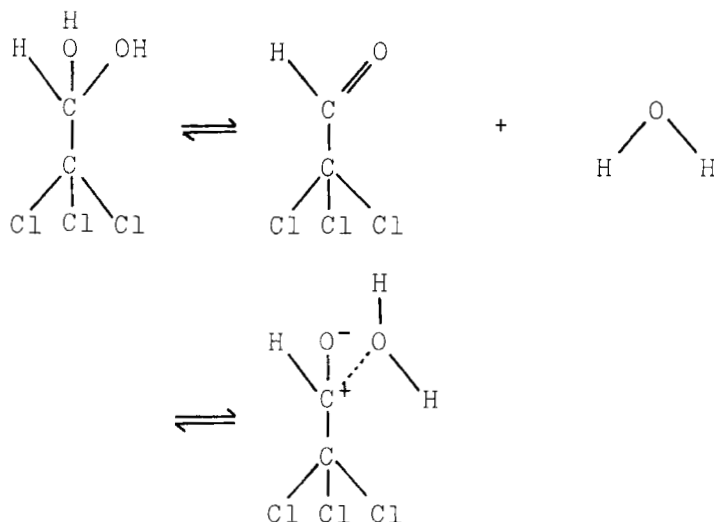
1.2 Appearance, Color, Odor, Taste

Colorless crystals with a pungent, but not acrid, odor. Has a pungent and rather bitter taste. Volatilizes slowly on exposure to air.

2. Physical Properties2.1 Spectra2.11 Infrared Spectrum

An understanding of the infrared spectrum of chloral hydrate should be approached from a study of the spectrum of chloral, which has been described in some detail in the literature^{1,2,3}. Chloral hydrate has been shown to have a gem-diol structure by Raman spectra⁴ and by N.M.R.⁵ Comparison of the infrared (mull) spectra of the compound and of its deuterated analog¹⁷¹ indicates that the two OH groups are not equal. This is confirmed by solution spectra^{17,18,23,168,171} and Ogawa²³ suggests that chloral hydrate exists in solution in an equilibrium state, which may be represented as follows:-

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Near infrared studies⁶ on a solution of chloral hydrate show the existence of a labile equilibrium between the gem-diol and a dimolecular 1:1 complex of the aldehyde and water. It is thought that the gem-diol structure results from the electronic effect of the halogens transmitted through the molecule, rather than from intramolecular hydrogen bonding between the halogens and the hydroxyl hydrogens.

The near-infrared spectrum of chloral hydrate in chloropicrin (trichloronitromethane) has been recorded⁶. Infrared spectra recorded in Nujol^{7,171} and in potassium bromide^{8,9,10} show the absence of the carbonyl band at 1765 cm⁻¹, but spectra recorded in solution (carbon tetrachloride^{7,10,11,169} benzene⁷, and chloroform¹⁷⁰,) show the formation of the carbonyl band, indicating some dissociation. Piguet and Jacot-Guillarmod⁷ concluded that, in carbon tetrachloride solution, chloral hydrate dissociates into chloral and water, eventually forming a distinct stable hemihydrate. However, Ekeblad¹¹ found that solutions containing up to 0.15g chloral hydrate per 100 ml of carbon tetrachloride could be prepared without dissociation.

In the OH region, dissociation causes a decrease in the OH peak extinctions and the appearance of a new OH peak from water at 3720 cm^{-1} . As expected, there is a shift in the OH stretching frequency from the crystalline state (3330 cm^{-1}) of chloral hydrate to dilute solution $17,18,23$. In the latter case, two OH peaks (of approximately similar intensity) appear at 3580 and 3615 cm^{-1} . However, freshly prepared solutions in the concentration range 10^{-2} to 10^{-3}M show no significant variations in the molar extinctions at the two peak maxima and no water or chloral peaks are observed. The infrared spectra of the Squibb House Standard Lot 76147 in mineral oil and in KBr are presented in figures 1 and 2¹².

2.12 Raman Spectrum

The Raman spectra of both chloral^{13,13,172,221,222,223,224} and chloral hydrate^{13,172,222,223,224} have been reported. Fonteyne²²³ found that the incremental addition of water to chloral produced a new Raman spectrum, whereas the lines due to chloral disappeared slowly. The line at 1760 cm^{-1} , corresponding to the $\text{C} = \text{O}$ displacement, is not entirely absent in chloral hydrate, suggesting the existence of an equilibrium.

The most recently reported spectra are those of Matsushima¹³.

2.13 Ultraviolet Spectrum

The u.v. spectrum of chloral hydrate has been recorded in a number of solvents.^{14,225} In solution in water or in dioxan containing a few percent of water, the hydrate is so stable that no carbonyl absorption is detectable. However, if the crystalline hydrate is dissolved in cyclohexane, it dissociates sufficiently to give a considerable absorption at $290\text{m}\mu$.¹⁴ (giving an ϵ value of 38.3).

A spectrum of the u.v. absorption of a solution of chloral hydrate in 95% ethanol (6.0g. in 25ml of solution)¹⁵ shows strong end absorption above 240m μ .

2.14 Nuclear Magnetic Resonance Spectrum

The N.M.R. spectrum obtained depends on the position of the chloral \rightleftharpoons chloral hydrate equilibrium. Chloral gives a simple spectrum (CDCl₃), the aldehyde proton absorbing at δ 9.1. Chloral hydrate, however, consistently gives a peak at δ 9.1, plus other peaks whose positions are more variable, for hydroxyl absorptions are greatly influenced by temperature and concentration¹⁶ (see fig.3).

A broad-line N.M.R. study⁵ has been used to define the structure of chloral hydrate as a gem-diol, distinct from the alternative structure containing molecular water, as in gypsum. At 200°K, chloral hydrate exhibits a slight triplet structure due to the three-spin CH(OH)₂ group which is substantiated by the second moment of the hydrogen resonance (8.9 ± 0.5 gauss²). The molecular hydrate structure would give a second moment greater than 15 gauss², whereas with the gem-diol structure, a second moment of about 3.8 gauss² would be expected. The figure obtained confirms the gem-diol structure, but it also follows that a large proportion of intermolecular broadening must be present, which could arise if the crystal involved extensive hydrogen bonding.

2.15 Nuclear Quadrupole Resonance Spectrum

The N.Q.R. spectra of ³⁵Cl in chloral hydrate and chloral deuterate have been recorded in the temperature range 77 to 323°K.^{17,18,19,20} (Similar spectra of ³⁷Cl have also been recorded²⁰). The frequencies of the quadrupole lines obtained by independent workers^{17,19,20} are in

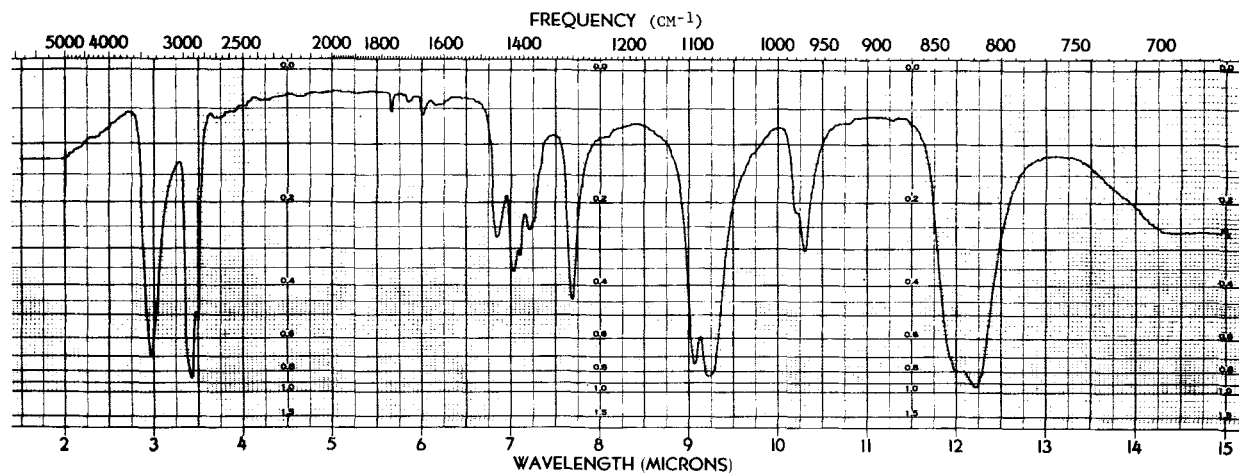


Fig. 1. Infrared spectrum of chloral hydrate,
Squibb House Standard (Mineral Oil Mull).

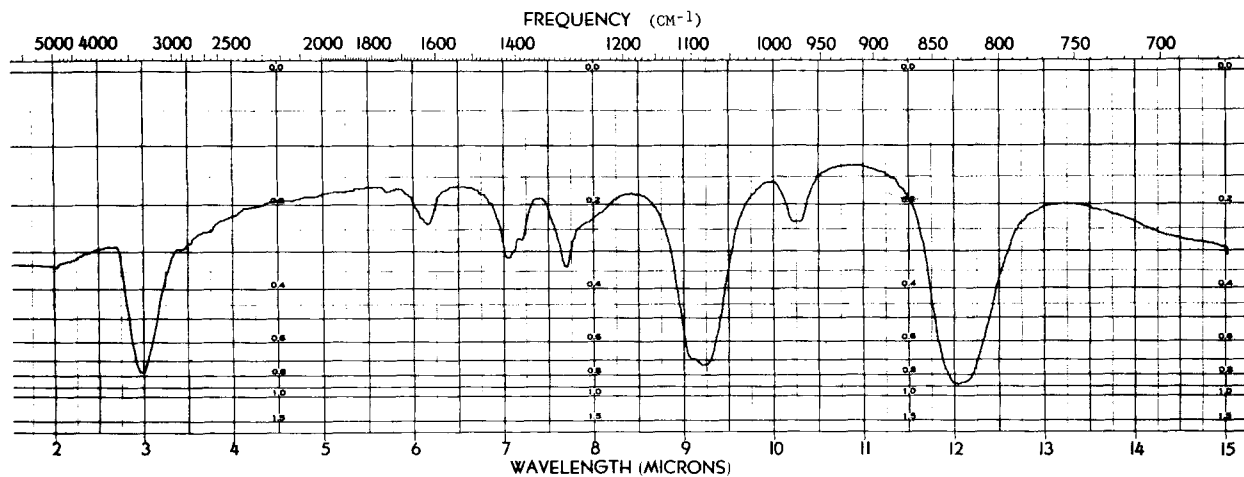


Fig. 2. Infrared spectrum of chloral hydrate,
Squibb House Standard (Potassium Bromide Disc).

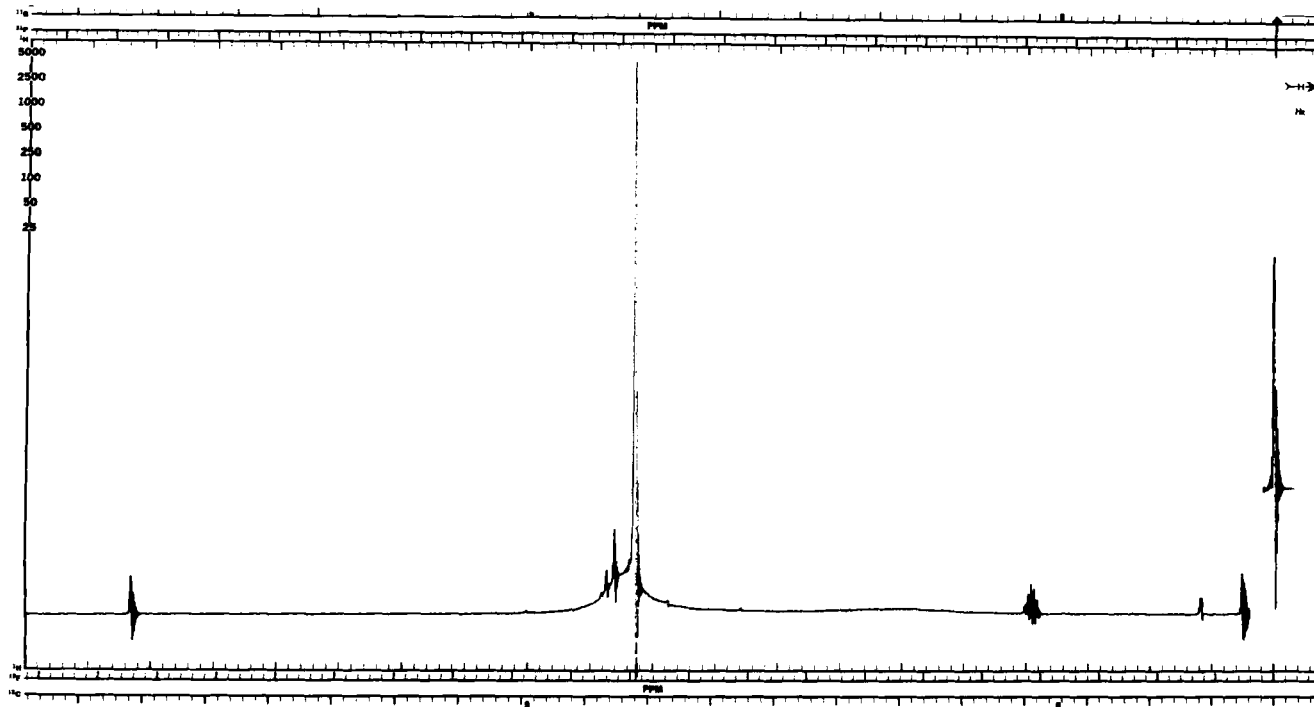


Fig. 3. N.M.R. spectrum of chloral hydrate,
Squibb House Standard, in deuterioacetonitrile.

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good agreement, and show that two of the lines are separated by only about 80 KHz whereas the third is about 1.25 MHz lower. This larger difference suggests a different C-Cl bonding for one of the Cl atoms.

Other studies ³⁰⁴ on the temperature dependence of the spin-lattice relaxation time for chloral suggest the crystallographic non-equivalency of the CCl_3 groups.

2.16 Mass Spectrum

The mass spectrum of chloral hydrate has been recorded on an MS-9 instrument ²⁴. As expected, chloral hydrate undergoes thermal dehydration at the source temperature (200°C), giving the highest mass detected as the molecular ion of chloral. The fragmentation pattern from this spectrum, which is given in Table 1, differs from that obtained by Reyes et al.²⁵ (with a Hitachi Perkin Elmer RMU-6D instrument) in the detection of additional peaks at m/e 118, 117, and 110.

TABLE 1
Fragmentation pattern produced in
mass spectral examination of chloral hydrate

m/e	Ion	produced by loss from molecular ion of chloral of
146	CCl_3CHO^+	-
118	CHCl_3^+	CO
117	CCl_3^+	CHO
111	CCl_2CHO^+	Cl
110	CCl_2CO^+	HCl
83	CCl_2H^+	Cl + CO
82*	CCl_2^+	Cl + CHO
47	CCl^+	CHO + Cl_2

* Most abundant peak

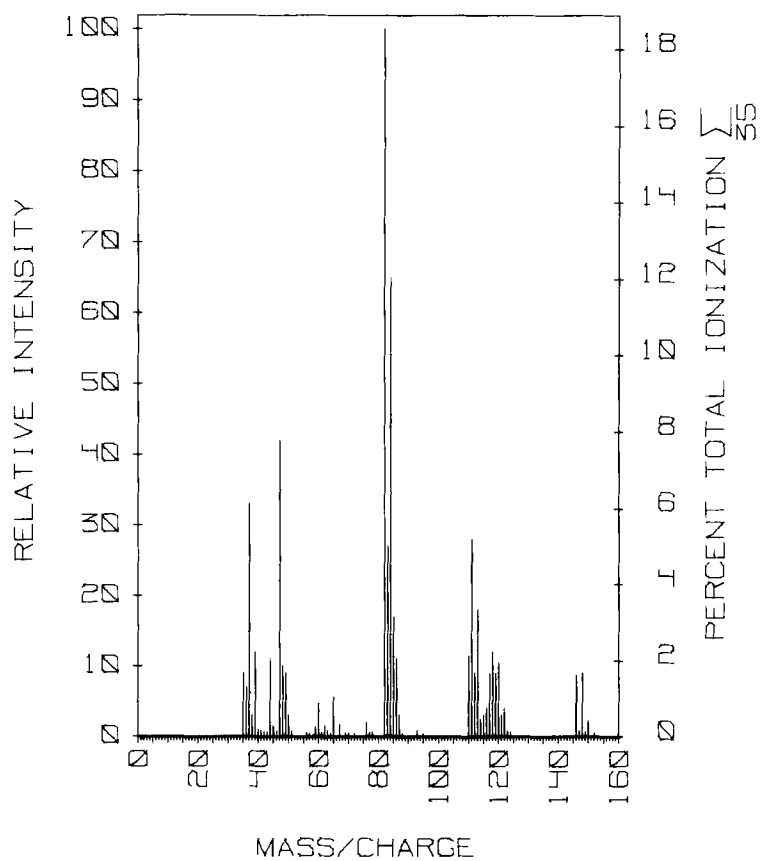


Fig. 4. Mass spectrum of chloral hydrate, Squibb House Standard.

2.17 Electron Spin Resonance Spectrum

Electron irradiation induces paramagnetic species in crystalline chloral hydrate which can be characterized by E.S.R. Spectrum²⁵. Two different intensity sequences have been observed thought to correspond to the $\text{CCl}_3\text{C}(\text{OH})_2$ and the $\text{CCl}_2\text{CH}(\text{OH})_2$ radicals.

2.2 Physical Properties of the Solid2.21 Basic Thermal Constants

The following have been reported:-

Heat and Free Energy of Formation¹¹¹ (for the crystalline state)

$$\Delta H_f = -132.2 \text{ (kg. cal. per mole)}$$

Heat of Vaporization (ΔH_o)

131.9 cal/g (at 96°C) by Lange¹¹¹

115.5 cal/g by Jacobson³⁰³

Latent Heat of Fusion

33.0 cal/g by Gehloff¹¹⁵

37.8 cal/g by Jacobson³⁰³

Specific Heat (Lange¹¹¹)

State	Temperature	Specific Heat
Crystalline	32°C	0.213
Liquid	55 to 88°C	0.470

Melting range and boiling point are variable, depending on dissociation and, thus, on such factors as the rate of heating.

Melting ranges as different as 46-47°C¹¹² and 59-60°C¹¹³ have been quoted in the literature, but the figure of 57°C given by the Merck Index¹¹⁴ appears to be the most representative.

Boiling point has been recorded by Lange¹¹¹ as 96.3 (764)°C and by the Merck Index¹¹⁴ as 98°C.

The Ebullioscopic Constant for chloral hydrate is given ¹²² as 2.28.

2.22 Differential Thermal Analysis and Differential Scanning Calorimetry

D.T.A. (open container) of the Squibb House Standard of chloral hydrate¹² gave a single endotherm at 59°C. D.S.C.¹¹⁶ (sealed container) of a sample of B.P. quality chloral hydrate similarly gave a single endotherm at 58°C. Separate experiments³⁰⁵ with U.S.P. grade chloral hydrate gave a single, well-defined melting endotherm at 56 to 57°C and a boiling point endotherm that varied from 105 to 125°C, depending on the amount encapsulated. Samples of chloral hydrate were heated to specified temperatures above the melting point, maintained for various periods at the temperature and then allowed to cool. Re-programming of the cooled samples gave unreproducible results that failed to show the presence of a discrete polymorphic form.

Dilatometric measurements¹¹⁷ that indicate a phase transition occurring at 32°C are in agreement with the much later¹¹⁸ report of an enantiotropic transition occurring at about 32°C (on cooling),^{301, 302} as observed with the polarizing microscope. The transition is not the same¹¹⁸ in the reverse direction (i.e. on raising the temperature). D.T.A. examination of chloral hydrate (room temperature to 70°C) as reported by Ogawa²³ detects the transition into a high-temperature form at 52.6°C, melting occurring between 55.0 and 64.5°C.

2.23 Loss on Drying and Thermo-gravimetric Analysis

Heated in open containers from room temperature to its melting point, chloral hydrate dissociates slowly, with volatilization of the products. The vapor pressure of chloral hydrate has been examined by several authors.^{117, 122, 300.}

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In the temperature range 15.65 to 38.50°C, a smooth vapor-pressure curve was obtained,¹¹⁷ rising from 6.12 to 26.90mm. α - bromonaphthalene. A separate source¹²² gives the vapor pressure of chloral hydrate at 20°C as 12.7mm. Hg.

2.24 Optical Characteristics

Chloral hydrate exists in the form of large monoclinic plates (hexagonal tabular crystals)^{23,114,118}. A less stable high-temperature form, obtained on crystallization of the melt^{19,23}, or from chloroform solution by evaporation²³, exists in a needle-like crystal form. Density of crystalline solid¹²² at 20°C is 1.908.

2.25 X-Ray Diffraction

The crystal and molecular structure of chloral hydrate has been investigated by x-ray diffraction^{23,119,120,121,312}. The crystal structure dimensions obtained by all three authors^{23,119,121} are in agreement, within the limits of experimental error. The crystal habit is monoclinic and belongs to the $P2_1/C$ space group, with four molecules in a cell of the dimensions:-

$$a = 11.50 \pm 0.03 \text{ \AA}$$

$$b = 6.04 \pm 0.02 \text{ \AA}$$

$$c = 9.60 \pm 0.03 \text{ \AA}$$

$$\beta = 120 \pm 0.5^\circ$$

The results of studies of molecular structure are less conclusive,^{19,21,22} and the results originally obtained by Kondo and Nitta²² were found to be in error by Ogawa²³, who repeated the work and reported bond lengths and bond angles. From these results, Ogawa suggested that the molecule contains two bifurcated bonds. However, the C-Cl bond lengths given by Ogawa have since been disputed by Milia and Hadjoudis²¹ on the basis of their N.Q.R. studies, which

suggest that only one hydrogen-bonded Cl exists.

An x-ray powder diffraction pattern has been recorded⁸⁵ for a batch of Chloral Hydrate U.S.P. (see Fig.5.). Ogawa²³ gives powder-diffraction patterns for both the normal hexagonal crystal plates of chloral hydrate and for the high-temperature needle crystals.

2.26 Neutron Diffraction

Neutron diffraction studies conducted by Brown¹²³ have yielded additional data about bond lengths and bond angles in chloral hydrate.

2.27 Polymorphism

Schill⁹⁴ rejected the possibility of enantiotropy or the existence of isomeric forms of chloral hydrate. However, he was able to prepare a hemihydrate (m.p. 95°C) reproducing the work of Meyer and Dulk¹²⁴. Piguet and Jacot-Guillarmod⁷ more recently prepared a "hemihydrate" that gave a well-defined infrared spectrum different from those of chloral hydrate or of a mixture of chloral and chloral hydrate.

Naveau¹¹⁸, rejecting Schill's conclusions, presented data supporting the existence of a dynamic isomerism between two stereo-isomers of unknown configuration.

Allen¹⁷, in an N.Q.R. examination of chloral hydrate, noted the need to "age" for 3 months material freshly re-crystallized from a melt before it would give a satisfactory spectrum. Biedenkapp and Weiss⁹ investigated this phenomenon more thoroughly, reporting the existence of two crystal modifications (see section 2.15). The less stable modification(I) was produced by freezing melts or by heating chloral hydrate to at least 48°C for 0.5 hour.¹²⁵ Modification(I) changed slowly to the more stable modification(II) in about 2-3 weeks at room temperature, but no accelerated transition was found even at 32°C.

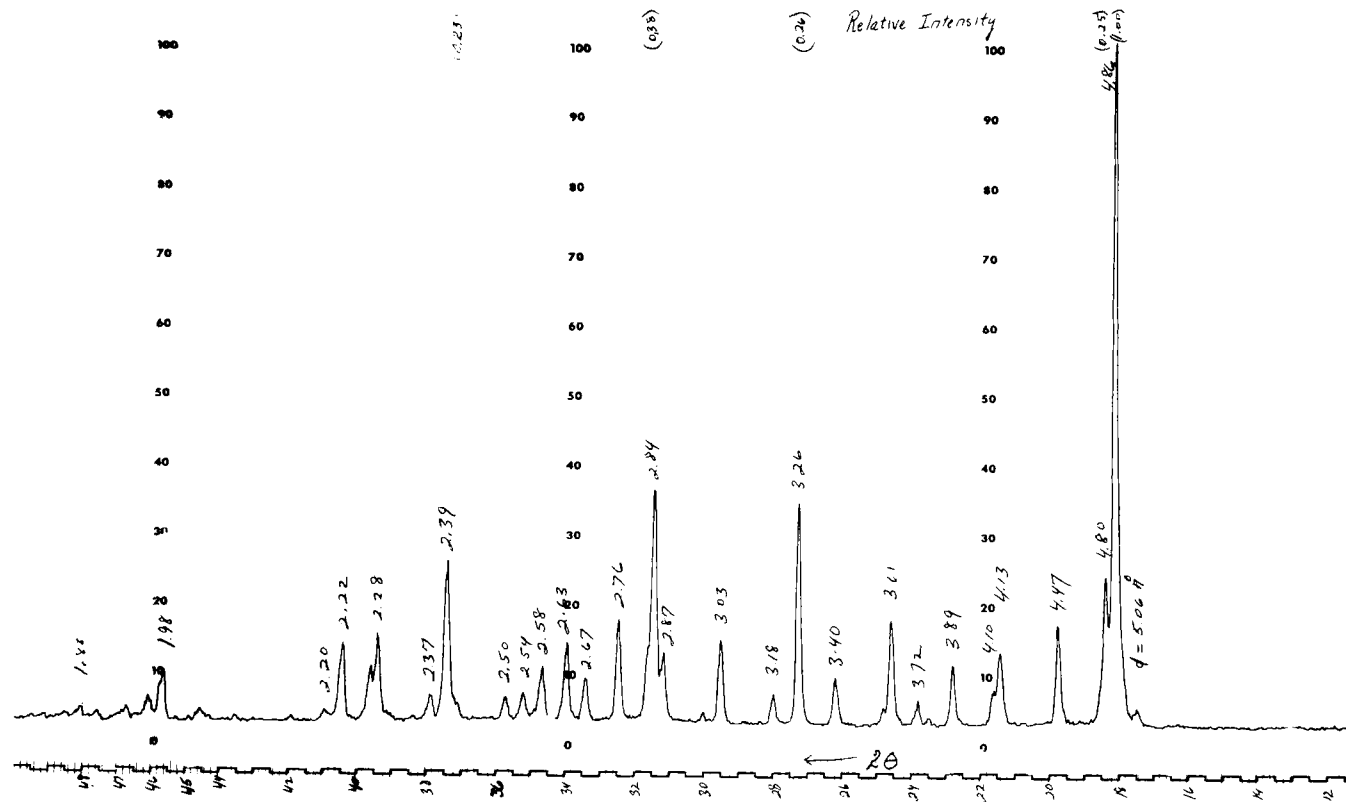


Fig. 5. X-ray powder-diffraction pattern of chloral hydrate, Lot T-102.

Ogawa²³ similarly detected the transition into a high-temperature form at 52.6°C (see Sections 2.22, 2.24 and 2.25). X-ray powder-diffraction patterns taken at different times after crystallization of the high-temperature form show that this phase changes slowly to the room-temperature phase.

2.3 Solubility

2.31 Solubility in Water

The solubility of chloral hydrate in water at 20°C is given by the B.P. (1968) as 1 part in 0.3 parts of water. However, the Merck Index¹¹⁴ gives the following aqueous solubilities:

<u>Temperature (°C)</u>	<u>Solubility(mg/ml)</u>
0	2400
10	3800
20	6600
30	10100
40	14300

2.32 Solubility in Water-Miscible Solvents

The solubility figures quoted for chloral hydrate in ethanol differ widely.

<u>Temperature</u>	<u>Volume of Ethanol (95%) required to dissolve 1g of Chloral Hydrate</u>	<u>Reference</u>
Room Temp.	1.3 ml	Merck Index
20°C	0.2 ml	B.P.1968 ⁹⁹

Equilibration of 15g of chloral hydrate with 1 ml of ethanol (95%) at 25°C for 24 hours resulted in a liquid system containing about 2 to 3 g of solid chloral hydrate¹³⁷. A parallel study with methanol gave similar results, but only about 1 g of solid remained¹³⁷. Results obtained with other water-miscible solvents are as follows:-

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<u>Solvent</u>	<u>Temperature</u>	<u>Solubility</u> (mg chloral hydrate per ml solvent)	<u>Reference</u>
Glycerol	Room Temp.	ca.1600	114
Acetone	-	Freely soluble	114
Methyl Ethyl Ketone	-	Freely soluble	114

2.33 Solubility in Water-Immiscible Solvents

<u>Solvent</u>	<u>Temperature</u> (°C)	<u>Solubility</u> (mg chloral hydrate per ml solvent)	<u>Reference</u>
Diethyl Ether	Room Temp.	ca. 660	114
Diethyl Ether	20	> 1000	99
Chloroform	20	155	138
Chloroform (ethanol-free)	25	380	137
Chloroform	20	ca.330	99
Chloroform (contg.2% ethanol)	25	525	137
Chloroform	Room Temp.	500	114
Carbon Di- sulfide	Room Temp.	11.6	114
Hexane	25	44.4	137
Heptane	25	39.2	137
n-Decane	25	33.0	137
Pet. Ether	Room Temp.	Sparsingly soluble	114
Carbon Tetra- chloride	Room Temp.	Sparsingly soluble	114
Benzene	Room Temp.	Sparsingly soluble	114
Toluene	Room Temp.	Sparsingly soluble	114

2.34 Solubility in Oils of Pharmaceutical Interest

<u>Solvent</u>	<u>Temperature</u> (°C)	<u>Solubility</u> (mg chloral hydrate per ml solvent)	<u>Reference</u>
Olive Oil	Room Temp.	ca. 710	114
Olive Oil	25	830	137
Castor Oil	25	1040	137
Corn Oil	25	990	137
Liquid Paraffin	25	13.3	±37
Turpentine	Room Temp.	Sparsingly soluble	114

2.4 Physical Properties of Solutions and Melts2.41 Heats of Solution and Dilution

The heat of solution of chloral hydrate in methanol has been determined microcalorimetrically as about -1.12Kcal/mol .¹⁰ Schill⁹⁴ measured the temperature rise on dissolution of chloral and melted chloral hydrate in water. In the same paper, he describes the temperature change observed on dissolving chloral hydrate in water, and compares it with those produced by chloral hemihydrate (see section 2.2) and by chloral hydrate melts that had been rapidly cooled to room temperature immediately before dissolution.

Schill⁹⁴ suggests that in a melt of chloral hydrate several hydrates of different structures may exist. By calculation from Schill's figures, an approx. heat of solution for chloral hydrate in water is -0.78Kcal/mol . Measurements of the heat of dilution of chloral hydrate aqueous solutions have been made⁹⁵. No Nernst temperature coefficient is found for these solutions.

2.42 Dissociation and pH

The dissociation of chloral hydrate in cyclohexane has already been mentioned in section 2.13. Dissociation of dilute aqueous solutions of chloral hydrate was reported in the same paper¹⁴.

The ionization constant, pK_a , was obtained by measuring the pH of buffered solutions of chloral hydrate⁹⁶. The mean of 25 experiments gave $pK_a = 10.04$, which differs considerably from the value of 11 obtained by Euler and Euler⁹⁷ and is closer to $pK = 9.77$, derived indirectly from kinetic measurements⁹⁸.

The dissociation of chloral hydrate in various organic solvents (CCl_4 , benzene etc.) has been studied by infrared spectroscopy⁷ (see section 2.11). Studies of the near infrared spectra⁶ have also been used to calculate equilibrium and rate constants for the dissociation of solutions of chloral hydrate.

2.43 Surface Tension

Teitel'baum et al.¹⁰⁰ give a complete picture of the surface tensions obtained with aqueous solutions of chloral (conc. range 2 to 100%) within the temperature range 0 to 75°C (at 5-°C intervals). These authors have also given attention to the temperature coefficient, which, when plotted against mol.% of chloral, shows a maximum at 50 mol.%, corresponding to the formation of chloral hydrate, and two minima at approx. 18 and 81 mol.%, respectively. Ferroni et al.⁵⁶ have also reported values for the surface tension of chloral hydrate solutions that range from 59.5 for 1 molar to 76.0 (dyne/cm.) at infinite dilution. Surface tension measurements made on solutions of chloral hydrate in diethyl ether, acetone, or benzene³⁰ indicate weak compound formation in these systems.

The surface tension of chloral hydrate melts³¹⁴ in the temperature range 53 to 100°C is expressed (in dynes/cm.) by the equation $\sigma = 44.6 - 0.110(t - 53^\circ)$. The contact wetting angle of its solid phase is $180^\circ 40' \pm 1.5^\circ$.

2.44 Ultrasonic Absorption

The variation of ultrasonic velocity and absorption with temperature has been recorded for chloral hydrate melts (for 7 MHz emission, velocities range from 1215 m/sec. at 52°C to 1125 m/sec. at 75°C)^{101,102}. The ultrasonic absorption coefficients (α/V^2) for chloral hydrate, when compared with those of a mixture of chloral and water at corresponding temperatures, show that the effect of dissociation brought about by increasing temperature is to reduce the absorption values.

Solutions of chloral in non-aqueous solvents^{27,31} show absorption-coefficient curves that pass through a maximum in the cases of associated solvents such as methanol and ethanol, corresponding to the formation of a molecular compound³⁰⁸.

2.45 Viscosity

The viscosities of chloral hydrate melts obtained by use of an Ostwald Viscometer have been reported¹⁰¹ as:-

<u>Temperature (°C)</u>	<u>Shear Viscosity (η/ρ) C.S.</u>
52	10.10
to	to
75	3.40

From the temperature coefficient of these viscosities, the activation energy of shear viscosity of chloral hydrate (melt) has been calculated as 10.6 Kcal/mol.

The viscosity of solutions of chloral

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in various hydroxylated organic solvents (aliphatic alcohols, benzyl alcohol, phenols, etc.), has been plotted against chloral concentration showing maxima where compound formation occurs.^{28,33,34,75,76,77,78,79,80,81}

2.46 Cryoscopy

The freezing-point depression caused by chloral hydrate in aqueous^{41,226} and alcoholic^{26,70} solutions has been described.

Van Rossem²²⁶, studying the freezing-point curves of chloral and water found evidence for three different hydrates: a "semihydrate" (1 mole of water to 2 moles of chloral), a monohydrate (1 mole of each), and a heptahydrate (7 moles of water to 1 mole of chloral). The heptahydrate had a sharp melting point at -1.4°C . Eutectic temperatures of 51°C with azobenzene and of 40°C with benzil have been reported³¹³.

2.47 Refractive Index

The refractive index of a chloral hydrate melt (at 60.0°C) rises with time⁹⁴, eventually reaching a constant value at 1.4807 (i.e. molar refraction M_R at 60°C is 29.47). The refractive index values obtained with a series of binary systems of chloral and various solvents (including water, ethanol, and benzene)⁷⁴ indicate the formation of an association compound between chloral and each solvent.

2.48 Radiolysis

Aqueous solutions of chloral hydrate, when irradiated with X-rays, gamma rays, or beta rays, give rise to hydrochloric acid as the end product of a chain reaction^{25,103} to¹¹⁰.

3. Molecular Complexes

3.1 Types of Known Complexes

Chloral forms a range of molecular complexes or adducts (mainly in a 1:1 ratio) with alcohols,^{26 to 31,79,92,93} diethylether,^{30,31} benzene,^{30,31} acetone,^{30,31} meprobamate,³⁷ tetracycline,³⁸ oxytetracycline,³⁸ acetaminophen,²⁹⁹ and others^{32 to 36,306.}

Similarly, chloral hydrate has been shown to form complexes (mainly 1:1) with acetyl glycinamide,^{39,40,41} betaine,^{44,45,46,47} caffeine,^{48,49} diazepam,^{50,312} 5,5-dimethylhydantoin,^{52,307} phenacetin,^{43,55} phenazone,^{41,56 to 61,} glycine,¹⁷⁴ glucose¹⁷⁶, urea^{53,68} and others.^{32,42,43,48,49,51,53,54,62-67,69,173,175,219.}

3.2 Structure of Complexes

A number of the molecular complexes of chloral and chloral hydrate have been shown to be hydrogen-bonded association compounds. The techniques employed in the examination of these complexes are summarized in Table 1.

TABLE 1

<u>Techniques used in the characterization of complexes of chloral and chloral hydrate</u>	
<u>Technique</u>	<u>References</u>
Cryoscopy	26,29,51,55,63,64,66,70,71,72,73
Differential Thermal Analysis	62
Differential Scanning Calorimetry	10,83,84
Heats of Solution	10,49
Refractive Index	74,86
Surface Tension	30,43,56
Viscosity	28,33,34,75-81
Infrared Spectroscopy	10,41,82
Raman Spectroscopy	57
X-ray Diffraction	10,85,312
Ultrasound	27,31
Hot-stage Microscopy	86

Chloral hydrate and phenazone form both

(1:1) and (2:1) molecular complexes. The structure of the (1:1) complex has been studied by infrared⁴¹ and Raman⁵⁷ spectroscopy, in addition to a surface-tension⁵⁶ study that examined both complexes.

The i.r. and Raman data suggest a strongly hydrogen-bonded complex, with the association at the carbonyl oxygen of the phenazone and not at the nitrogen. Infrared studies of the complex in carbon tetrachloride solution have been used to calculate a stability constant for the complex (1:1), and cryoscopic measurements on dilute aqueous solutions show the molecular compound to be completely dissociated in water⁴¹.

The crystal and molecular structure of the (1:1) molecular complex of chloral hydrate and bromodiazepam has been determined by x-ray diffraction studies³¹².

4. Synthesis and Purification

Chloral hydrate was first obtained by Liebig (1832) from the reaction of water with chloral, the latter obtained via the chlorination of ethanol. Industrially, chloral is prepared by passing chlorine into cooled ethanol (either absolute or 95 percent) to form the hemiacetal of trichloroacetaldehyde, from which chloral is liberated by treatment with sulfuric acid.

It has been found, however, that the hemiacetal may be hydrolyzed^{268,269} by the addition of water, giving chloral hydrate and alcohol.

The over-all reaction may be represented by the equation:-

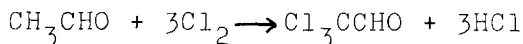
$$\text{EtOH} + 4\text{Cl}_2 + \text{H}_2\text{O} \longrightarrow \text{Cl}_3\text{CCH}(\text{OH})_2 + 5\text{HCl}$$
 Other process modifications include the replacement of ethanol with ethanol-acetic acid mixtures in the chlorination step²⁷⁰, with recycling of the less volatile byproducts, such as chloral acetate, chloral alcoholate, butylchloral, and

trichloroacetic acid.

Crude chloral is separated by fractionation from volatile byproducts such as ethyl chloride, ethyl ether, ethylene dichloride, and alcohol, and the chloral "cut" is taken between 93 and 98°C. This material¹⁹⁰ contains 2 to 3% water-insoluble material containing ethyl dichloroacetate, ethyl trichloroacetate, and trichloroacetal. The technical chloral separated from the oil contains acetic acid (0.2 to 0.8%), dichloroacetaldehyde (2 to 5%), ethyl dichloroacetate (about 0.3%), ethyl trichloroacetate (about 0.6%), and traces of chloral alcoholate¹⁹⁰.

The chloral hydrate formed by treating the chloral with an appropriate quantity of water may contain traces of the above impurities and also trichloroacetic acid (oxidation product) or trichloroethanol (reduction product). The crude chloral hydrate may then be purified further by recrystallization from an organic solvent such as benzene.

An alternative industrial route to chloral is by the chlorination of acetaldehyde or paraldehyde^{268,271}



Alternative laboratory preparations include the reaction of ethyl formate with sulfuryl chloride at 170°C ²⁷² and the reaction of chloroacetylene with sodium hypochlorite solution and saturated boric acid solution²⁷³. The preparation of high-purity chloral has been achieved²²⁸ by heating metachloral at 180-200°C.

5. Degradation

5.1 Degradation of Solid Chloral Hydrate

5.11 Volatilization

Chloral hydrate crystals, left open to

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the air, volatilize slowly as chloral and water. Condensation of the mixed dissociated vapors gives back chloral hydrate⁷. Chloral hydrate in sealed containers will only volatilize until equilibrium vapor pressure has been attained⁸⁹.

Volatilization may be determined by measurement of weight loss¹⁷³, by G.L.C. assay²⁵⁶ or by G.L.C. assay of the ullage gases⁸⁹ of the container.

5.12 Chemical Degradation

Degradation of chloral hydrate occurs in the presence of an excess of free oxygen to form phosgene, CO₂, and HCl, but only after a considerable lag-phase (52 days)²²⁷, unlike anhydrous chloral, which decomposes much more rapidly²²⁷.

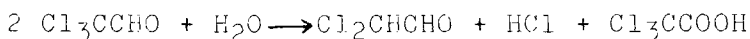
Exposure of chloral hydrate to sunlight in the absence of oxygen generates no significant levels of gaseous degradation products, even after 160 days²²⁷.

Traces of water may cause the degradation of chloral hydrate to dichloroacetaldehyde, trichloroacetic acid and hydrochloric acid; traces of alkali lead to the formation of chloroform and formic acid^{217,91}.

5.2 Degradation of Chloral Hydrate in Solution

5.21 Action of Light

It has been proposed^{217,218} that neutral aqueous solutions of chloral hydrate decompose by an oxidation-reduction process.



Exposure of solutions to light greatly accelerates this process, as may be demonstrated by measure-

ment of the formation of free hydrochloric acid.

5.22 Action of Heat

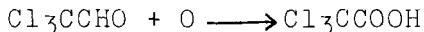
On warming, neutral and slightly acidic aqueous solutions of chloral hydrate appear to degrade to trichloroacetic acid, dichloroacetaldehyde, and hydrochloric acid²¹⁷.

5.23 Ultrasound (Sonocleavage)

Exposure of aqueous solutions of chloral hydrate to high-energy ultrasound (1MHz; 1.8kV) has been shown²²⁰ to cause a degradation of the chloral hydrate that follows zero-order kinetics. The main degradation products are hydrochloric acid and dichloroacetaldehyde.

5.24 Autoxidation and Polymerization

Anhydrous chloral has been shown to undergo autoxidation in air²²⁷ via both of the following reactions and possibly others.



The action of light appears to be necessary for at least the initial formation of the peroxide catalyst. Anti-oxidants, such as hydroquinone, resorcinol, and α - and β -naphthols, decrease the oxidation rate, whereas complete protection is given by aniline, diphenylamine, and related compounds²²⁸. In addition to the gaseous products of autoxidation, a solid polymer thought to be metachloral is formed. Chloral hydrate does not appear to undergo autoxidation unless it first dissociates to chloral and water²²⁷.

Strong sulfuric acid causes the polymerization of chloral to form α - and β -parachlorals²³¹, which have been shown to be stereoisomers of the cyclic trimer of chloral^{232,233}. More dilute sulfuric acid produces the amorphous metachloral

231, which may be a noncyclic polymer of chloral hydrate⁶.

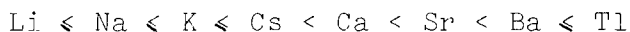
The anionic polymerization of chloral has been studied at -78°C , with Bu Li and sodium naphthalene²³⁴ to ²³⁶ used as initiators.

5.25 Action of Bases

Chloral hydrate decomposes in dilute aqueous solutions of sodium hydroxide⁹⁸. This first-order reaction is catalysed by the hydroxyl ions, by the chloralate ion and by water. It proceeds via an intermediate bivalent chloralate ion which decomposes further to give chloroform and the formate ion.

Further studies by Pfeil et al.²³⁷ indicate that in the presence of excess base the reaction is first order and the rate increases almost linearly with base concentration. However, for equimolar concentrations the reaction becomes second order and with excess chloral hydrate the hydrolysis of the Cl_3C - group becomes the predominant reaction.

The addition of solvents of low dielectric constant (such as dioxan) increases the rate of activity towards the degradation of chloral hydrate to vary for a range of hydroxides as follows:-



Tuchel et al.^{238,239,240} have also examined the kinetics of the degradation of chloral hydrate in solutions containing sodium hydroxide, borax, disodium hydrogen phosphate and sodium phenobarbital. In addition, these authors examined the stabilizing effect of certain colloids (gum arabic, agar-agar, and gelatin) on mixed solutions of chloral hydrate and sodium phenobarbital.

5.26 Radiolysis

Andrews and Shore¹⁰⁷ reported that aqueous solutions of chloral hydrate were decomposed on irradiation with x-rays (50-200kv.) to form hydrochloric acid and a smaller amount of a weakly ionised acid. Many other authors^{25,103 to 106,108 to 110,241,242} have examined the irradiation of chloral hydrate solutions with x-rays, gamma-rays or beta-rays. In all cases it is suggested that the chloral hydrate decomposes to hydrochloric acid via a chain reaction.

5.27 Action of Microorganisms

The degradation of chloral hydrate in soils has been examined^{243,244} and the results suggest that microorganisms participate in the degradation in conjunction with chemical and photochemical processes.

5.28 Incompatibilities

Chloral hydrate has been shown to be chemically incompatible with many compounds. It is probable that it is incompatible with all substances forming complexes with it or with chloral (see Section 3.1, Tables 1 and 2), with most bases (see Section 5.25) and with many compounds bearing hydroxyl groups.

The following table lists substances known to be incompatible with chloral hydrate (other than known complex formers).

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Substances Showing Chemical Incompatibility with Chloral Hydrate

<u>Substance</u>	<u>Reference</u>	<u>Substance</u>	<u>Reference</u>
Caustic Alkalies	245,237,98	Lactose	87
Alkaline Earths	245,246	Glucose	176
Alkali Carbonate	245,246	Peroxides	229,230
Soluble			
Barbiturates	245,239,240	Metal Alkyls	234,249
Borax	238	Tannin	245
Disodium			
Hydrogen	238	Iodides	245
Phosphate		Perman-	
		ganates	245
Piperidine	247		
Quinine Salts	245	*Stearic Acid	88
Diuretin	246	*Sucrose	88
Phenol	245	*Cora Oil	88
Mannitol	87	*Sesame Oil	88
		*Peanut Oil	88
p-Methyl			
Cyclohexanol	248	*Olive Oil	88
Menthol	248	*Castor Oil	88
iso-Menthol	248	*Lanolin	88
Borneol	248	*Carbowax	88
iso-Borneol	248	*Cetomacro-	
		gol 1000	88
Camphor	245	*Calcium	
		Phosphate	88
Thymol	245		

* Substances causing degradation of the chloral hydrate moiety of a chloral hydrate complex.

5.29 Interaction with Starch

Chloral hydrate may be reacted with starch to form a product soluble in cold water²⁵¹ or a clear antiseptic adhesive²⁵⁰ coacervate²⁵²,²⁵³.

5.3 Stabilization of Chloral Hydrate

Chloral hydrate may be protected from autoxi-

dation and polymerisation by the incorporation of small quantities of antioxidants such as aniline or diphenylamine²²⁸. Following similar lines, chloral may be stabilized by the addition of 0.2 to 1% formamide or dimethylformamide²⁵⁴. Similarly 0.1% tetra-alkylthiuram disulfide or 0.1% to 0.2% azobisisobutyronitrile has been used²⁵⁵. Chloral has also been stabilized in the presence of transition metal polymerization initiators such as Fe, Sb or Ni by the addition of 0.05 to 0.5% ϵ (or δ) caprolactam³⁰⁵.

6. Chemical Properties

6.1 Identity Tests

Chloral hydrate may be identified by its melting point and its ability to yield chloroform when reacted with sodium hydroxide solution^{15,99,127}.

It may be identified by measurement of physical parameters, such as infrared spectrum¹², laser Raman spectrum²⁵⁷, or G.L.C. retention time^{195,199}.

Chloral hydrate gives rather unsatisfactory conventional aldehydic derivatives²⁵⁸, such as oxime (m.p. 56°C), semicarbazone (m.p. 90°C (d)), and 2,4-dinitrophenylhydrazone (m.p. 131°C), but forms many complexes that have clearly defined melting points (see Section 3.1).

Chloral hydrate gives a strong Fujiwara color reaction (see Section 6.22), which may be used as an identity test^{199,259}. Similarly, it gives positive color tests with the diazo-reagent²⁶⁰, the indole reagent for aldehydes²⁶¹, and the aniline²⁶² reagent. However, it does not give a positive test with Schiff's reagent and some other aldehyde reagents²⁶³. A color test claimed to distinguish chloral hydrate from other trichloro-compounds not possessing a carbonyl group involves reaction with resorcinol in dilute alkali.^{8,275}

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Other color tests employ as reagents, codeine-sulfuric acid³¹⁰, thiobarbituric acid³¹¹ and 1-phenyl-3-methyl-5-pyrazolone³¹¹. Several spot tests with a sensitivity of 1 µg or less have been described^{264,265}.

6.2 Methods of Analysis

6.21 Volumetric/Titrimetric Procedures

Crude chloral, obtained by the liquid-phase chlorination of ethanol, has been assayed¹²⁶ by a procedure involving the volumetric measurement of the chloroform formed after hydrolysis with strong KOH solution.

Chloral hydrate is easily decomposed by alkali into chloroform and alkali formate. This reaction is used stoichiometrically to determine chloral hydrate purity by measuring the amount of an excess of 1N sodium hydroxide consumed in the reaction (titration with 1N sulfuric acid to phenolphthalein)^{15,99,127,128,152 to 166}. Alternatively, the formate generated in the reaction may be determined by reaction with an excess of iodine (0.1N), followed by back titration with 0.1N sodium thiosulfate¹²⁹ (or hydrazine)¹³⁵ or, alternatively, by reaction with 0.1N potassium permanganate and potassium iodide, followed by titration of the liberated iodine^{130,131}. In the latter procedure, sodium carbonate is used in place of sodium hydroxide because it is less likely to hydrolyze the chloroform produced.

More vigorous hydrolysis of chloral hydrate will convert the chloroform (produced initially) into ionic chloride, which can then be determined by Volhard titration. Procedures for this hydrolysis have been described that use ammonium persulfate¹³² or the reflux of chloral hydrate with aluminium powder and aqueous acetic acid¹³³.

The oxidation of chloral hydrate (to trichloroacetic acid)¹⁶⁷ by acid-dichromate¹³⁴,

followed by back titration of the excess dichromate with a stabilized Mohr's salt solution (to phenylanthranilic acid), has been used as a quantitative procedure¹³⁴.

Titration of chloral hydrazone with sodium metavanadate solution has been described¹³⁶.

Other titrimetric methods include the potentiometric¹⁴⁸ titration of chloral hydrate and the acidimetric¹⁴⁹ determination of chloral, following its oxidation with silver oxide.

6.22 Spectrophotometry

The formation of an intense red color by the action of pyridine on chloral hydrate in the presence of alkali was first described by Fujiwara¹³⁹. The reaction is not specific, similar colors being produced by other organic trihalogeno-compounds (chloroform, trichloroacetic acid etc.).

The color produced is very sensitive to the reaction conditions used, which have to be critically defined for the color intensity to be used as a quantitative parameter.

The reaction has found much use in the determination of chloral hydrate in biological tissues and fluids, and a recent paper¹⁴⁰ describes the quantitative determination of chloral hydrate in the range 6 to 60 μ g.

The conditions of reaction have been studied extensively for the determination of chloroform and carbon tetrachloride, and the presence of acetone in the reaction mixture was found to improve the color¹⁴¹.

A number of other modifications of the Fujiwara procedure have been described¹⁴² to ¹⁴⁶.

Friedman and Cooper¹⁴⁷ found that the

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chloral hydrate reaction product also absorbs at 370m μ , with an intensity three to four times that of the absorption at 540m μ . This aspect, and the use of the reaction to differentiate chloral hydrate from trichloroethanol, which gives a product absorbing at 440m μ , have been examined thoroughly^{140,147}.

Feigl¹⁵⁰ noted that the color of the Fujiwara reaction product is discharged by acetic acid, and that the addition of benzidine then causes the formation of a violet color. He suggests that the products of the Fujiwara reaction are Schiff bases of glutaconic aldehyde, formed by ring opening of the pyridinium adducts formed between pyridine and the polyhalogenated compounds. Hydrolysis of the original Schiff bases and treatment with benzidine yields the same derivative irrespective of the nature of the polyhalogenated compound. This modification of the Fujiwara reaction has been made quantitative¹⁵¹ and although it is unspecific for chloral hydrate it gives a five to eight-fold increase in sensitivity over that of Friedman and Cooper¹⁴⁷ and is less susceptible to interference than using the peak at 370m μ .

Stehwein and Kühmstedt¹⁵² describe a more specific color reaction for chloral hydrate which as a quantitative procedure has a sensitivity limit of about 50 μ g/ml of chloral hydrate. In this reaction chloral hydrate is quantitatively converted to chloral oxime with hydroxylamine hydrochloride. The chloral oxime is then condensed with 2,6-diaminopyridine in N hydrochloric acid to give a cherry-red pyri-isatin dye (λ max. 530m μ). Archer and Haugas¹⁷⁷ developed a colorimetric procedure for the specific determination of chloral hydrate (up to 150 μ g/10ml sample solution). This procedure uses the color (605m μ) produced by chloral hydrate on reaction with quinaldine ethiodide in alkaline (ethanolamine) isopropanol.

The specificity of the procedure has been well examined¹⁷⁷ and a wide range of compounds have been screened for interference in the reaction¹⁸³.

Only chloral/chloral hydrate hemiacetal compounds and molecular complexes have been found to interfere. The procedure offers good accuracy and precision and has found considerable application^{9,90,178 to 182,297,298,309} as a stability assay procedure for chloral hydrate and its complexes.

Other colorimetric assay procedures have been described^{183 to 188,296}.

6.23 Ion-Exchange and Column Chromatography

Chloral hydrate is not absorbed on ion exchange resins by an ion exchange mechanism but it has been found to be absorbed on a cation-exchange resin (KU-1) by a process of molecular sorption¹⁸⁹.

The nonabsorption of chloral hydrate on anion exchangers has been used to develop an assay procedure for it in the presence of acetic, monochloroacetic, and trichloroacetic acids, and sodium trichloroacetate^{189,244}.

Column chromatography has also been used to determine the purity of chloral¹⁹⁰.

6.24 Paper and Thin-layer Chromatography

Paper chromatography has been used²⁷⁴ to determine trichloroacetic acid in chloral hydrate.

Thin-layer chromatography has been used to separate chloral hydrate from amylchloride and dichloroethane, from admixture with monochloroacetic acid and the acetate ester of monochloroethanol¹⁹¹ and from formaldehyde³¹¹.

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Simple solvent-screening studies have been carried out on microchromatoplates (Kieselgel GF₂₅₄ layers 250 μ thick) with the results reported in Tables 1 and 2¹⁹². In this study¹⁹², the spots of chloral hydrate were located by spraying with a reagent (resorcinol, 5g, potassium hydroxide, 5g), in absolute ethanol, 50ml, followed by gentle warming.

Chloral hydrate appears as a pink spot on a white background (cf.ref.10).

TABLE 1

Approximate Rf values for chloral hydrate

<u>Solvent System</u>	<u>Rf Value</u>	<u>Appearance of Spot</u>
Cyclohexane	0.0	Compact
Carbon tetrachloride	0.0	Compact
Benzene	0.1	Compact
Dichloromethane	0.1	Sl.spread
Chloroform	0.3	Compact
Acetone	0.9	Sl.spread
Ethanol (95%)	0.9	Compact
n-Propanol	0.9	Sl.spread
Diethyl ether	1.0	Compact
Ethyl acetate	1.0	Compact
Methyl ethyl ketone	1.0	Compact
Dioxan	1.0	Compact
n-Butanol	1.0	Sl.spread

TABLE 2Approximate Rf Values for Chloral Hydrate in
Multicomponent Solvent Systems

<u>Solvent System</u>	<u>Ratio</u>	<u>Rf Value</u>	<u>Appearance of Spot</u>
Benzene/Methanol/ Ammonia (0.880)	(90:10:1)	0.4	Compact
Benzene/Ethanol/ Ammonia (0.880)	(48:16.5:2.5)	0.8	Compact
Methanol/Ammonia (0.880)	(100:1.5)	0.9	Compact

The volatility of chloral considerably reduces the usefulness of quantitative T.L.C.

6.25 Vapor-Phase Chromatography (V.P.C., G.L.C.)

Early workers^{193,194} converted the chloral hydrate to chloroform by reaction with alkali and then subjected the chloroform to quantitative G.L.C.

The direct injection of chloral hydrate solutions has been described more recently by a number of authors, and a summary of their systems is given in Table 3 (see also references 200 and 201).

Methods of quantitation have ranged from the use of standard plots and direct comparison with a standard, to the use of internal standards, such as chlorobutanol^{197,198} and chloroform¹⁹⁷.

The sensitivity obtained by use of a flame-ionisation detector is only slightly greater than that obtained with the modified Fujiwara procedure described by Friedman and Cooper¹⁴⁷.

TABLE 3

G.L.C. (V.P.C.) Determination of Chloral Hydrate

Column Support	Column Stationary Phase	Column Temperature (°C)	Retention Time (min)	Detector System	Type of Determination	Reference
Chrom R 60/80 (mesh)	20% Apiezon L	100	11-12	Katherometer	Forensic	195
Firebrick 60/80	20% Carbowax 20M	130	7-8	Electron Capture	Forensic	195
Chromosorb W 60/80	10% Apiezon L	Programmed 1.5/min from 60 to 90 28/min from 90 to 160	-	F.I.D.	Metabolic	196
Chromosorb W 60/80	20% Carbowax 20M	100	ca.1	F.I.D.	Metabolic	197
Chromosorb W 60/80	20% Carbowax 20M	125	-	Electron Capture	Metabolic	197
Chromosorb W 60/80	15% FFAP	105	ca.1	Electron Capture	Forensic	198
Glass Beads	0.6% Apiezon L	75	23ml at a rate of 37ml/min.	Electron Capture	Metabolic	199

TABLE 3 (cont'd)

Column Support	Column Stationary Phase	Column Temperature (°C)	Retention Time (min)	Detector System	Type of Determination	Reference
Column Pak T 40/60	5% Silicone Fluid 550	80	5.5 - 6	Katherometer	Purity	12
Gas-Chrom Q	3%JXR	50	ca. 1	Electron Capture	Vapor Pressure	89
Poropak Q		160	21	Electron Capture	Stability Assay for Degradation Products	91
Chromosorb 60/80	20% Dow-Corning Silicone 200	60	-	Katherometer	Study of Dissociation in Vapor Phase	7

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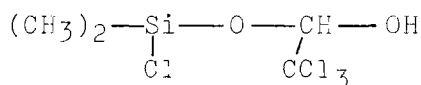
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However, the use of an electron-capture detector greatly increases the sensitivity, but linear response obtained is only over a small range. Jain et al.¹⁹⁸ reported a working range of 0.6 to 2.5×10^{-9} chloral hydrate (i.e. non-linear above 1.5×10^{-11} moles). Salmon and Heyes⁸⁹ obtained similar results using a Pye-Unicam Ni⁶³ detector, saturation of the detector being observed at about 10^{-11} moles giving a working range between 10^{-13} and about 10^{-12} moles.

G.L.C. procedures have also been developed for the determination of impurities in chloral hydrate^{12,15,91,127,196,197}.

Reaction of chloral hydrate has been reported with some of the more common reagents used in the preparation of derivatives for G.L.C. Diazomethane²⁰² gives an unstable intermediate, $\text{Cl}_3\text{CCH}(\text{OH})\text{CH}=\text{N}_2$, at $<0^\circ\text{C}$ (in Et_2O), which yields 1,1,1,5,5,5-hexachloro-2,3-epoxy-4-hydroxypentane or 1,1,1-trichloro-2,3-epoxy-propane.

Condensation of chloral hydrate²⁰³ with Me_2SiCl_2 in pyridine/chloroform yields a colorless viscous oil.



The reaction between chlorosilanes and chloral complexes containing nitrogen (e.g. di-chloral-urea) has also been examined²⁰⁴.

6.26 Refractometry and other physical parameters

Methods have been described for the quantitative determination of chloral hydrate using refractometry²⁰⁵ and surface tension¹⁵⁵ or measurement of specific gravity^{206,207}.

6.27 Polarography

The polarographic reduction of chloral

hydrate was first reported for solutions in 0.1N KCl by Neiman *et al.*²⁰⁸, who quoted a value of $E_{\frac{1}{2}}$ for chloral hydrate of - 0.8v. Federlin^{209,210} and his co-workers reported a single reduction wave ($E_{\frac{1}{2}}$ about - 1.6v) for chloral hydrate in tetramethylammonium buffer, which they ascribed to a diffusion-controlled process involving reduction of the hydrated molecule to dichloroacetaldehyde, which was considered to be non-reducible.

Elving and Bennett^{211,212}, after a very thorough examination of the polarographic reduction of chloral hydrate in a selection of ammonia, phosphate and borate buffers, disputed Federlin's findings. They reported that, in all cases, they had found dichloroacetaldehyde to give two kinetic-controlled waves (- 1.0v and - 1.7v). They advanced the theory that the chloral hydrate wave ($E_{\frac{1}{2}}$ about - 1.4v against S.C.E.) results from the synthesis of diffusion and kinetic reduction processes. Their theory is supported by polarographic and coulometric data showing the reduction of chloral hydrate to dichloroacetaldehyde hydrate, which then dehydrates by a kinetic process.

The dichloroacetaldehyde formed reduces to chloroacetaldehyde, then to acetaldehyde, and, finally, to ethyl alcohol and/or 2,3-dihydroxybutane.

The complete reduction sequence to acetaldehyde results in the one wave, but the reduction of the acetaldehyde produces a second wave ($E_{\frac{1}{2}}$ about - 1.7v) that is only clearly resolved in ammonia buffers. The variation of $E_{\frac{1}{2}}$ with pH and with the nature of the supporting electrolyte is summarized in Tables 4 and 5.

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TABLE 4

Effect of pH on the polarographic behavior of chloral hydrate at 25°C (from Elving and Bennett)

Buffer	pH	Conc. (mM)	Head (cm.)	- E _{1/2} (v)	n
Disodium phosphate - citrate	7.2	0.25	40	1.42	1.4
Ammonium chloride - ammonia	8.4	0.25	40	1.34*	1.9
Ammonium chloride - ammonia	9.1	1.0	80	1.36*	2.6
Borate-KCl	9.2	1.0	80	1.45	2.2
Borate-KCl	9.8	1.0	80	1.67	1.7

* In the case of ammonia buffers, a second wave is found at E_{1/2} about - 1.66v.

TABLE 5

Effect of supporting electrolyte on the polarographic behavior of chloral hydrate at 25°C (from Federlin²¹³)

Solvent	Supporting Electrolyte	pH	Conc. (mM)	-E _{1/2} (v)
Water	LiCl (0.1M)	-	1	1.635
	(CH ₃) ₄ NC1 (0.1M)	-	1	1.420
Dioxan/Water (50:50)	(CH ₃) ₄ N ⁺	6.7	1	1.345
	Li ⁺	9.6	1	1.600
	(CH ₃) ₄ NOH	13.0	1	1.520

Use of the polarographic reduction as a quantitative assay procedure is capable of selectively determining chloral hydrate in the presence of dichloro- and chloroacetaldehyde²¹².

A quantitative polarographic assay for chloral hydrate has been described²¹⁴ that util-

izes the reduction of chloral oxime at pH2 in a HCl -KCl buffer (two waves are obtained at approx. $E_{\frac{1}{2}}$ - 0.55 and - 1.20v.).

6.28 Determination of Water

The water content of chloral hydrate has been successfully determined by the Karl Fischer titration procedure²¹⁵ and by reaction with calcium carbide²¹⁶.

6.3 Radiolabelled Chloral Hydrate

Thin-layer chromatography of chloral hydrate after neutron irradiation gave several radioactive components¹⁹¹. Chloral hydrate - ($1-^{14}\text{C}$) and - ($2-^{14}\text{C}$) have been prepared by chlorination of labelled ethanol^{244,266} and used to monitor the degradation of chloral hydrate in soil²⁴⁴.

Chloral hydrate - t (^3H -labelled chloral hydrate) has been prepared²⁶⁷ by the Wilzbach technique, using 8Ci of ^3H (12mm.) and subjecting to Tesla excitation for 15 minutes. The specific activity of the product was 30 - 110 mCi/g.

7. Metabolism

The hypnotic properties of chloral hydrate were first described by Liebreich (1869)²⁷⁶. It is rapidly absorbed from the stomach^{18,245} and reaches an effective blood level within 0.5 hr. in man. Mackay and Cooper²⁸² suggest that the CNS depression that follows ingestion is mainly, if not entirely, due to its metabolite, trichloroethanol. Comparison of the efficacy of chloral hydrate and trichloroethanol supports this supposition²⁹³.

Trichloroethanol is produced²⁷⁹ by a reaction catalyzed by the enzyme, alcohol dihydrogenase^{286,292}, which takes place in the liver, whole blood, and various other tissues²⁸¹. In man, a major proportion of the trichloroethanol

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is then conjugated and excreted in the urine as urochloralic acid^{277,278,144,279,280,281,283} (trichloroethanol glucuronide), which has been characterized as 2',2',2'-trichloroethyl- β -D-glucosiduronic acid^{288,290}.

A variable proportion of chloral hydrate is oxidized in the liver and kidney by a DPN-dependent enzyme to give a CNS-inactive metabolite, trichloroacetic acid²⁸⁷. This is the main urinary metabolite found in man, but is minor to trichloroethanol (free plus conjugated) in the dog and rat¹⁴⁵.

A small proportion of the trichloroethanol may also be converted to trichloroacetic acid¹⁴⁶.

Chloral hydrate is not detected in the blood or urine in man^{146,197,198}, but is found in small amounts in the blood of the dog,¹⁴⁵ rat,¹⁴⁵ and mouse¹⁴⁰. The rate of metabolism of chloral hydrate is so rapid that free chloral hydrate was not detected in human blood in the period 5 min. to 6 hr. after the administration of 1g of chloral hydrate²⁹⁴ (sensitivity of analytical method 0.5 μ g chloral hydrate/ml blood). However, in the same²⁹⁴ individuals, trichloroethanol was still present in the blood (20 to 65 mg %) 6 hr. after dose.

The determination procedures for the levels of the metabolites in blood, urine, and tissue have centered around various modifications of the Fujiwara reaction¹⁴⁰. Procedures have been reported for the determination of trichloroethanol,^{143 to 147,284,285,296,289} trichloroacetic acid²⁸⁹, and urochloralic acid^{140,146,292}. Gas-liquid chromatography has also been used for the determination of chloral hydrate, trichloroethanol, and urochloralic acid¹⁹⁶ in blood, urine,^{197,199}, and tissue homogenate^{198,295}. Trichloroacetic acid has not been determined directly in this manner, but may be determined indirectly after its conversion to chloroform¹⁹⁷.

Urochloralic acid is first hydrolyzed to tri-chloroethanol by acid hydrolysis^{197,198,291} or by incubation with β -glucuronidase¹⁹⁷.

The glycone obtained by the enzymic hydrolysis of urochloralic acid has been identified¹⁹⁷ as D-glucuronic acid by T.L.C. on a layer of Silica Gel GF254, using the solvent system n-butanol/acetic acid/water (4:5:1) and visualization by charring.

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CLIDINIUM BROMIDE

Bruce C. Rudy and Bernard Z. Senkowski

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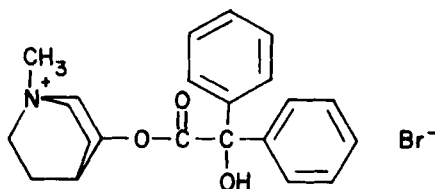
Analytical Profile - Clidinium Bromide

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1. Description

1.1 Name, Formula, Molecular Weight

Clidinium bromide is 3-hydroxy-1-methylquinuclidinium bromide benzilate.



$C_{22}H_{26}BrNO_3$

Molecular Weight: 432.36

1.2 Appearance, Color, Odor

Clidinium bromide occurs as a white or nearly white, almost odorless, crystalline powder.

2. Physical Properties

2.1 Infrared Spectrum (IR)

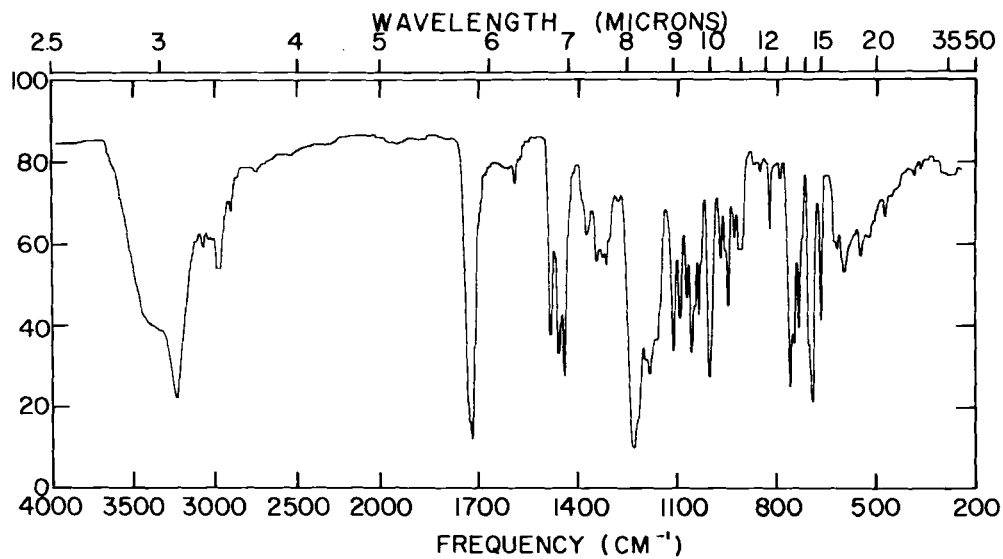
The infrared spectrum of clidinium bromide is presented in Figure 1 (1). The spectrum was measured with a Perkin-Elmer 621 Spectrophotometer in a KBr pellet containing 1.7 mg of clidinium bromide/300 mg of KBr. Table I lists the assignments for the characteristic bands in the IR spectrum (1).

Table I

Infrared Assignments for Clidinium Bromide

<u>Frequency (cm⁻¹)</u>	<u>Characteristic of</u>
3226	OH stretching vibrations
3059	aromatic CH stretching vibration
1726	C=O stretch of the ester
1595 and 1489	aromatic C=C stretching vibration
1238-1236	C-O-C stretch of the ester linkage
767 and 696	out of plane bending of 5 adjacent H's on benzene rings

Figure 1
Infrared Spectrum of Clidinium Bromide

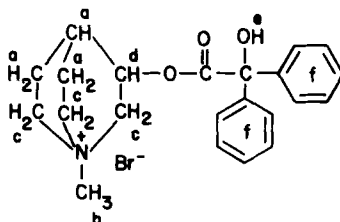


2.2 Nuclear Magnetic Resonance Spectrum (NMR)

The spectrum shown in Figure 2 was obtained in a Jeolco 60 MHz NMR by dissolving 54.3 mg of clidinium bromide in 0.5 ml of DMSO-d₆ containing tetramethylsilane as an internal reference (2). The spectral assignments are given in Table II (2).

Table II

NMR Assignments for Clidinium Bromide



Protons	No. of Protons Derived from Integration	Chemical Shift (ppm)	Multiplicity
a	5	1.4-2.4	Complex Multiplet
b	3	3.05	Singlet
c	6	3.1-4.3	Complex Multiplet
d	1	5.25	Complex Multiplet
e	1	6.86	Sharp Singlet
f	10	7.45	Singlet

2.3 Ultraviolet Spectrum (UV)

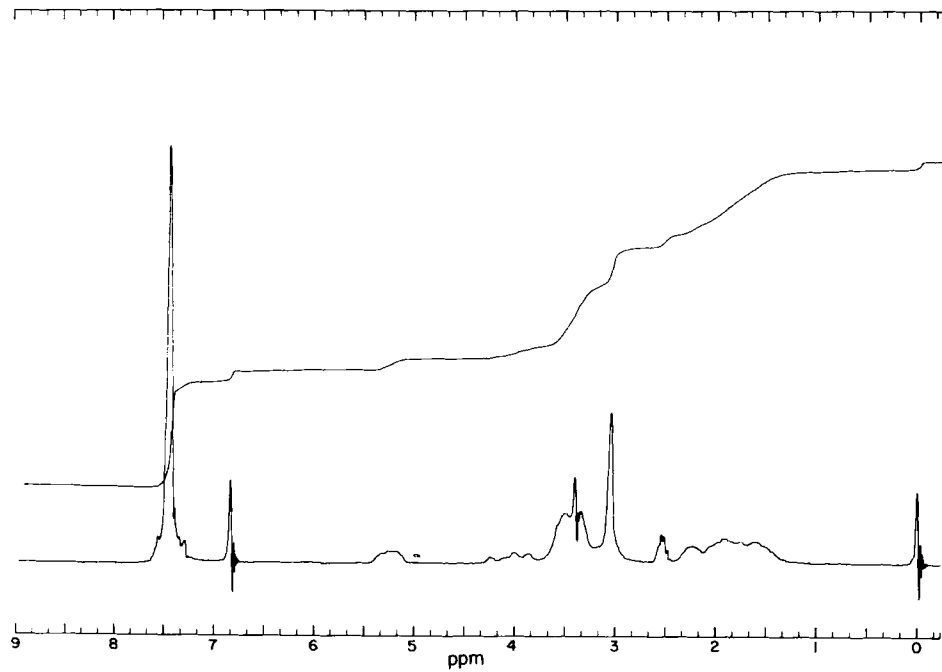
When the UV spectrum of clidinium bromide was scanned from 350 to 210 nm, two sharp maxima occurred at 257 nm ($\epsilon = 4.8 \times 10^2$) and 251 nm ($\epsilon = 4.3 \times 10^2$). No further maxima were reached between 240 and 210 nm but the absorbance was still rapidly increasing at 210 nm. The spectrum shown in Figure 3 was obtained from a solution of 50.735 mg of clidinium bromide/100 ml of water (3).

2.4 Fluorescence Spectrum

No fluorescence was observed for clidinium bromide in water, methanol, 0.1N HCl, or 0.1N NaOH when the sample was irradiated with polychromatic light (4).

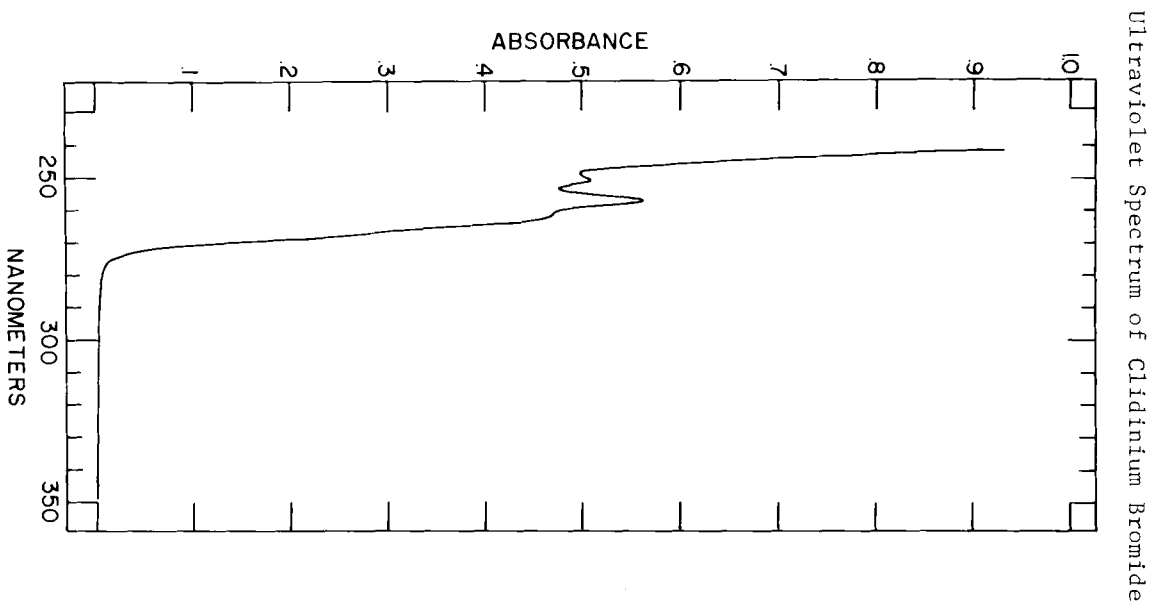
Figure 2

NMR Spectrum of Clidinium Bromide



CLIDINIUM BROMIDE

Figure 3



2.5 Mass Spectrum

The mass spectrum of clidinium bromide was obtained using a CEC 21-110 mass spectrometer with an ionizing energy of 70 eV. The output from the mass spectrometer was analyzed and presented in the form of a bar graph, shown in Figure 4, by a Varian 100 MS dedicated computer system (5). The highest mass observed at m/e 337 is a thermal breakdown product where CH_3Br was lost from the parent quaternary ammonium compound. The base peak at m/e 183 is due to the diphenylhydroxymethyl fragment. The rest of the peaks are due to a multitude of thermal breakdown products related to the parent clidinium bromide molecule (5).

2.6 Optical Rotation

Clidinium bromide exhibits no optical activity.

2.7 Melting Range

The melting range reported in the NF XIII for clidinium bromide is 240° to 244° using the class I procedure (6).

2.8 Differential Scanning Calorimetry (DSC)

The DSC scan for clidinium bromide is shown in Figure 5. The extrapolated onset of the melting endotherm occurs at 242.0°C when the temperature program was $10^\circ/\text{minute}$. The ΔH_f was found to be 9.2 kcal/mole (7).

2.9 Thermogravimetric Analysis (TGA)

The TGA performed on clidinium bromide showed no weight loss from ambient to 270°C . A single continuous weight loss occurred from 270° to 390°C equivalent to about 90% of the sample (7).

2.10 Solubility

The solubility data for clidinium bromide obtained at 25°C are given in Table III (8).

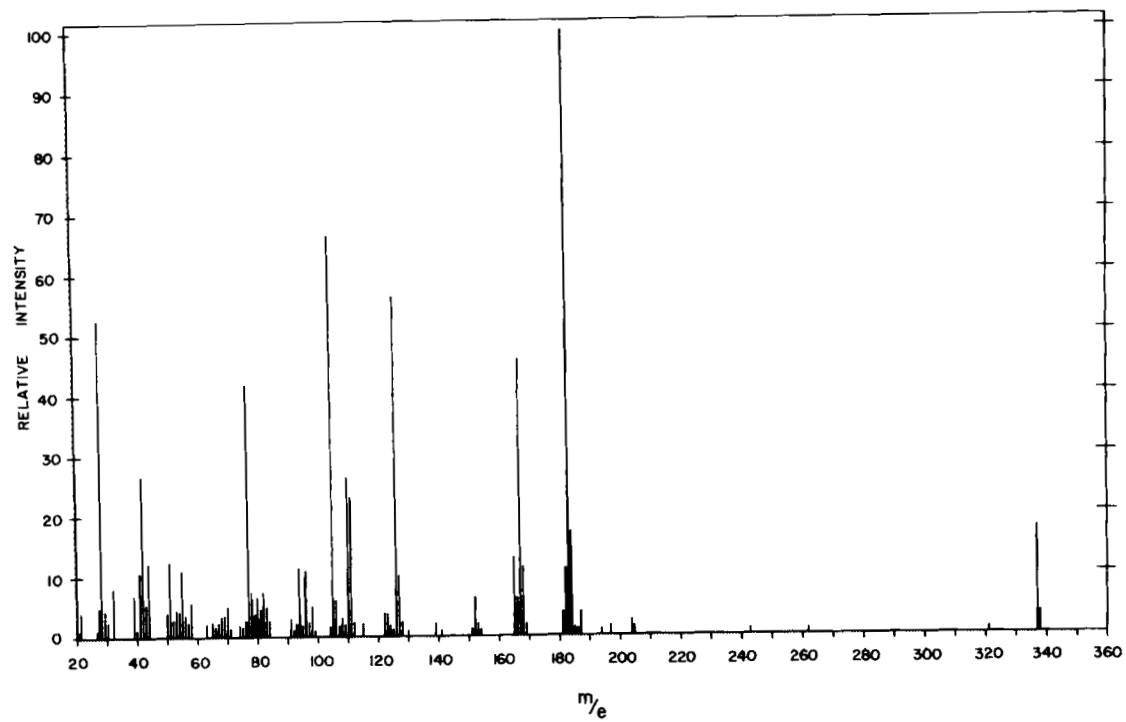
Table III

Solubility of Clidinium Bromide in Different Solvents

<u>Solvent</u>	<u>Solubility (mg/ml)</u>
3A alcohol	9.3
benzene	0.06
chloroform	1.4

Figure 4

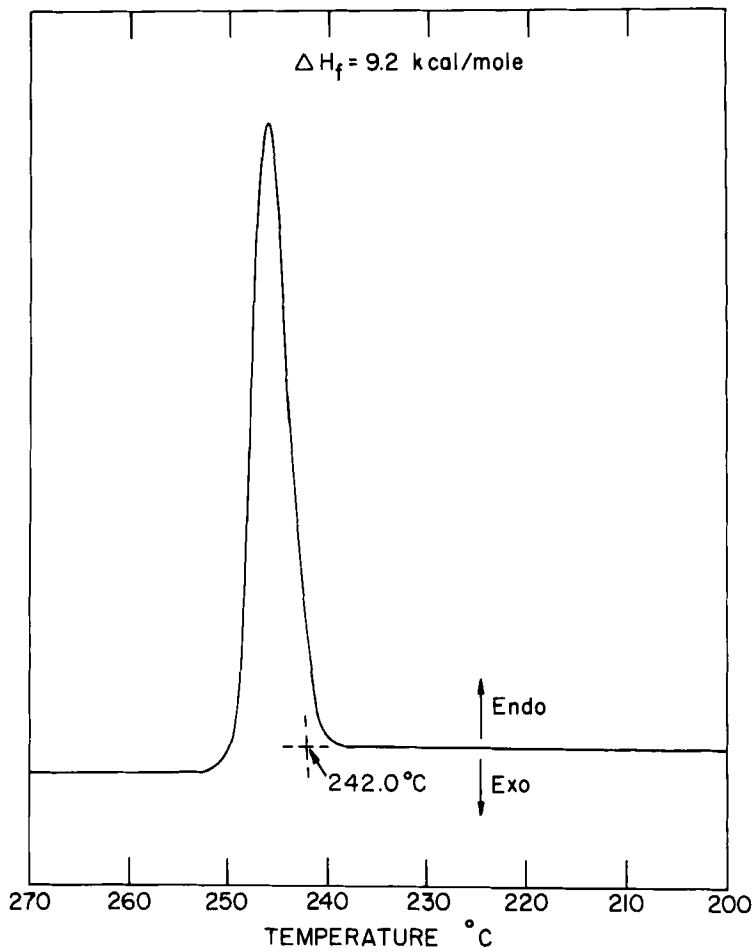
Mass Spectrum of Clidinium Bromide



CLIDINIUM BROMIDE

Figure 5

DSC Scan for Clidinium Bromide



CLIDINIUM BROMIDE

95% ethanol	26.4
ethyl ether	<0.05
methanol	102.4
petroleum ether 30°-60°	<0.05
2-propanol	0.6
water	82.7

2.11 X-ray Crystal Properties

The x-ray powder diffraction pattern of clidinium bromide is presented in Table IV (9). The instrumental conditions are given below.

Instrumental Conditions:

General Electric Model XRD-6 Spectrogoniometer

Generator:	50 KV-12-1/2 MA
Tube target:	Copper
Radiation:	Cu K_{α} = 1.542 Å
	0.1° Detector slit
	M.R. Soller slit
	3° Beam slit
	0.0007 inch Ni filter
	4° take off angle
Goniometer:	Scan at 0.2° 2 θ per minute
Detector:	Amplifier gain - 16 course, 8.7 fine
	Sealed proportional counter tube and DC voltage at plateau
	Pulse height selection E_L -5 volts; E_u - out
	Rate meter T.C.4
	2000 C/S full scale
Recorder:	Chart speed - 1 inch per 5 minutes

Samples prepared by grinding at room temperature.

Table IV

X-ray Powder Diffraction Pattern of Clidinium Bromide

2θ	$d(\text{\AA})^*$	I/I_o^{**}	2θ	$d(\text{\AA})^*$	I/I_o^{**}
8.66	10.2	6	29.32	3.05	13
9.42	9.39	13	29.60	3.02	11
10.52	8.41	40	30.21	2.96	11
11.10	7.97	60	30.82	2.90	17
12.36	7.16	43	31.38	2.85	6
14.26	6.21	45	32.28	2.77	10
14.70	6.03	15	33.10	2.71	6
15.55	5.70	3	33.65	2.66	24
16.18	5.48	73	34.15	2.63	23
16.82	5.27	14	34.98	2.57	11
17.20	5.16	19	35.55	2.53	4
17.72	5.01	13	35.86	2.50	7
18.26	4.86	48	36.88	2.44	11
18.80	4.72	16	37.20	2.42	6
19.18	4.63	5	37.80	2.38	3
19.56	4.54	32	38.30	2.35	6
19.94	4.45	6	39.20	2.30	9
21.14	4.20	14	39.46	2.28	8
21.64	4.11	25	40.05	2.25	6
21.96	4.05	37	40.75	2.21	3
22.40	3.97	44	41.50	2.18	7
22.72	3.91	22	42.03	2.15	2
23.13	3.85	35	42.58	2.12	4
23.38	3.80	70	43.25	2.09	2
24.10	3.69	32	43.68	2.07	8
24.76	3.60	35	44.84	2.02	15
25.50	3.49	17	45.48	1.99	4
26.35	3.38	25	46.80	1.94	3
26.92	3.31	100	47.60	1.91	3
27.48	3.25	3	48.50	1.88	11
27.61	3.23	3	48.90	1.86	7
28.25	3.16	11	49.19	1.85	4
28.27	3.11	5	50.19	1.82	3

* d - (interplanar distance) $\frac{n\lambda}{2 \sin \theta}$

** I/I_o = relative intensity
(based on highest intensity of 1.00)

3. Synthesis

Clidinium bromide is synthesized by the reaction scheme shown in Figure 6. 3-Quinuclidinol is reacted with methyl benzilate to form the ester. The ester is then reacted with methylbromide to form the quaternary salt, clidinium bromide (10).

4. Stability Degradation

Clidinium bromide is stable in aqueous solution between pH 2 and pH 8. In 6N HCl the ester bond gradually hydrolyzes at room temperature and in alkaline media rapid decomposition occurs (11). In formulations the drug has been found to be stable for at least five years (12).

5. Drug Metabolic Products

Clidinium bromide is metabolized to 1-methyl-3-hydroxy-quinuclidinium bromide which is the major form of the drug found in the urine of dog and man (11). Both the intact drug and the above mentioned metabolite are found in the feces of dog and man (11). Studies using ^{14}C indicate that the drug is not metabolized by N-demethylation (11).

6. Methods of Analysis

6.1 Elemental Analysis

The results from the elemental analysis are listed in Table V (13).

Table V

Elemental Analysis of Clidinium Bromide

<u>Element</u>	<u>% Theory</u>	<u>% Found</u>
C	61.12	61.30
H	6.06	6.13
N	3.24	3.14

6.2 Phase Solubility Analysis

Phase solubility analysis may be carried out using 2-propanol as the solvent. An example is shown in Figure 7 which also lists the conditions under which the analysis was carried out (8).

Figure 6
Synthesis of Clidinium Bromide

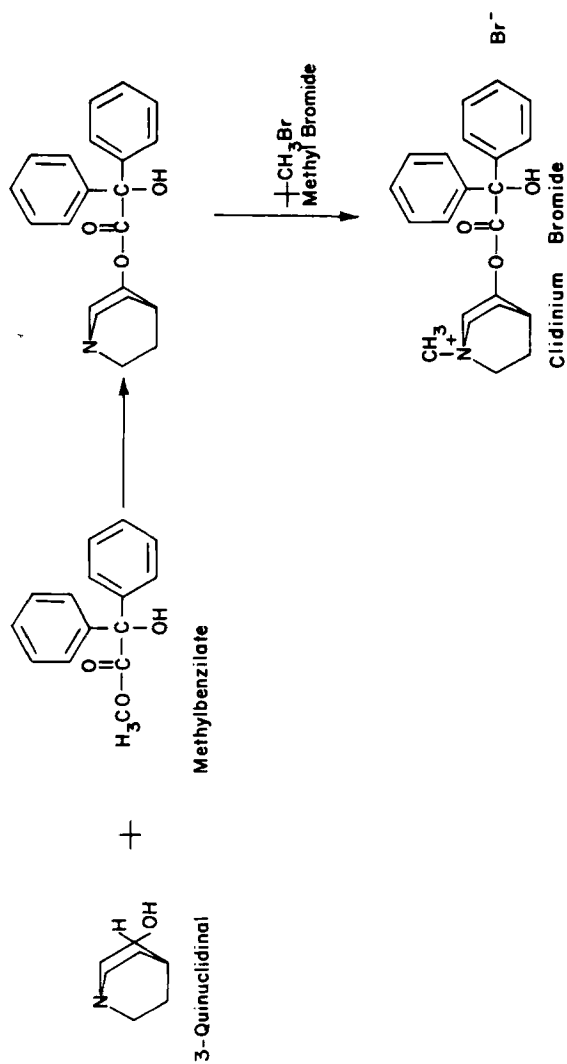
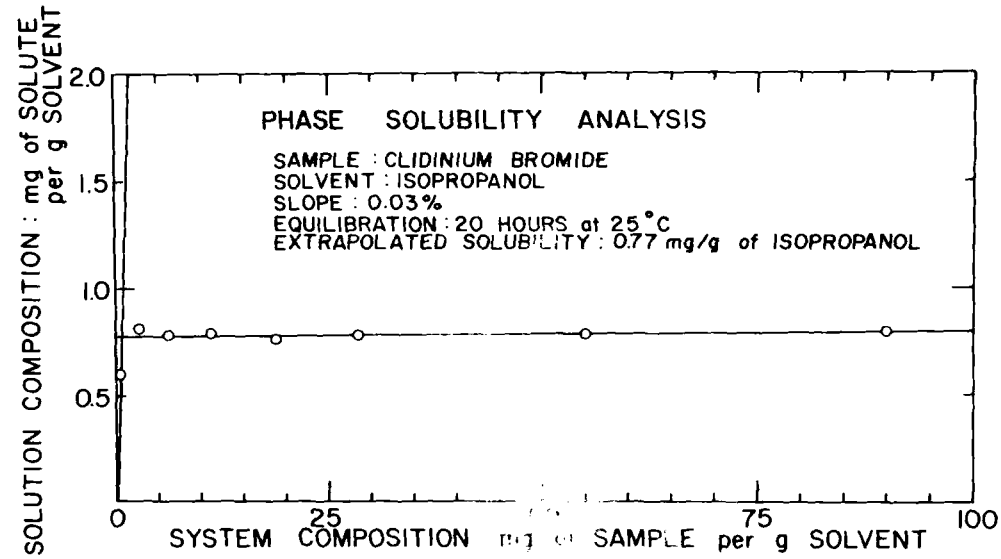


Figure 7



6.3 Thin Layer Chromatographic Analysis (TLC)

The following TLC procedure is useful for separating clidinium bromide from 3-hydroxy-1-methylquinuclidinium bromide and 3-quinuclidinyl benzilate (6). On two silica gel G plates that have been prewashed in the developing solvent (given below) and activated 15 minutes at 105°C, apply 2 mg of clidinium bromide sample in methanol. Develop the plates at least 10 cm in fresh developing solvent made of acetone:methanol:water:concentrated HCl (14:4:1:1). Remove the plates, dry at 105° for 10 minutes, cool and spray with the potassium iodoplatinate to detect any 3-quinuclidinyl benzilate (R_f 0.8) present and spray the other plate with Modified Dragendorff reagent to detect the clidinium bromide (R_f 0.5) and any 3-hydroxy-1-methylquinuclidinium bromide (R_f 0.4) present (6).

6.4 Direct Spectrophotometric Analysis

Direct spectrophotometric analysis may be carried out in water using the maximum at 257 nm. Due to the extremely narrow peak, however, narrow slit widths are required and great care must be exercised in locating the exact maximum.

6.5 Colorimetric Analysis

Clidinium bromide forms an ion pair complex with thymol blue in an aqueous solution buffered at pH 8.8. This complex can be extracted into chloroform and its absorbance read at the maximum at about 410 nm (14).

6.6 Titrimetric Analysis

The potentiometric titration with perchloric acid in dioxane is the method of choice to assay clidinium bromide (6). The sample is dissolved in glacial acetic acid, an excess of mercuric acetate is added, and the titration with 0.1N HClO₄ in dioxane is carried out. The equivalence point is determined potentiometrically. Each ml of 0.1N HClO₄ is equivalent to 43.24 mg of clidinium bromide.

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DEXAMETHASONE

Edward M. Cohen

Contents

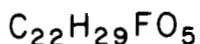
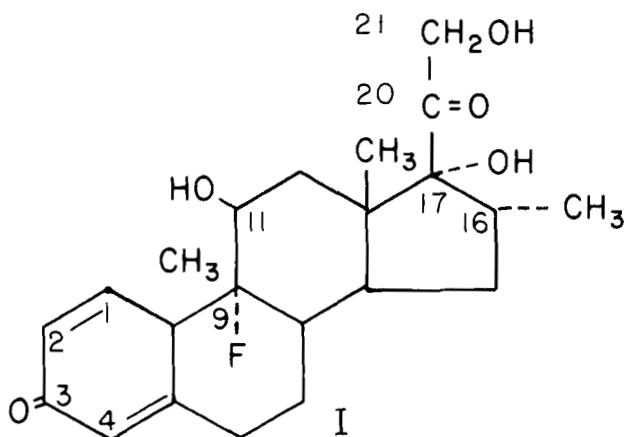
1. Description
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DEXAMETHASONE

1. Description

1.1 Name, Formula, Molecular Weight, Regulatory Status

Dexamethasone (I)* is 9 α -fluoro-11 β ,17 α ,21-trihydroxy-16 α -methylpregna-1,4-diene-3,20-dione. Other names for the compound include 9 α -fluoro-16 α -methylprednisolone and 16 α -methyl-9 α -fluoroprednisolone. The Merck Index lists three other chemical names for dexamethasone. Proprietary names for dexamethasone include Decadron, Dexacortisyl, Oradexon, Hexadrol and Deronil. A number of others are listed in the monograph for dexamethasone in the Merck Index.¹



Mol. Wt.: 392.45

Official monographs for dexamethasone are given in USP XVIII and B.P. 1968.

*Betamethasone is the 16 β -methyl configurational isomer of dexamethasone.

1.2 Appearance, Color, Odor

White to yellowish-white, odorless, crystalline powder.

2. Physical Properties

2.1 Crystal Properties

The optical crystallographic properties of dexamethasone reported for crystals obtained by crystallization of the steroid from 50% ethanol are as follows:²

System: orthorhombic	Dispersion: $r > v$ strong
Crystal Habit: Tabular	Refractive Indexes: $\alpha (a) = 1.559$
Optic Sign: +	$\beta (e) = 1.579$
Axial Angle: 50°	$\gamma = 1.649$
Optic Orientation: $xx \parallel a$	Density: 1.337
($c < a < b$)	$yy \parallel c$ Molar Refraction:
	$zz \parallel b$ Experimental = 99.64
	Calculated = 98.59

Two crystal forms of dexamethasone have been observed to date. The X-ray powder diffraction data for the two forms A and B are given in Table I.³ Because of variations encountered in peak intensity from sample to sample, no listing of the relative intensity of the bands is given. The two crystal forms have essentially the same solubility in water and essentially the same decomposition temperature. Form B is the more usually observed form. No solvates or polymorphic modifications of dexamethasone were reported by Mesley^{4,5} for dexamethasone crystallized from chloroform, acetone and ethanol using X-ray diffraction patterns and solid state I.R. spectra to evaluate samples.

2.2 Infrared Spectrum (I.R.S.)

Mesley reports the following band assignments for the solid state I.R.S. of dexamethasone (mineral oil mull).⁵

DEXAMETHASONE

TABLE I

X-Ray Powder Diffraction Pattern of Dexamethasone

Sample - Merck Standard #8415-76

Cu-K α Radiation

<u>2θ°</u>	<u>Form A</u>	<u>d^b</u>	<u>2θ°</u>	<u>Form B^a</u>	<u>d^b</u>
5.70		15.48	5.95		14.80
7.95		11.10	6.92		12.75
9.90		8.92	7.45		11.83
12.45		7.10	8.50		10.38
13.50		6.55	8.80		10.03
14.45		6.12	10.55		8.36
14.95		5.91	12.50		7.07
16.10		5.49	13.60		6.50
17.65		5.02	14.20		6.23
19.25		4.60	15.10		5.90
20.00		4.43	15.60		5.67
20.60		4.30	16.80		5.27
20.90		4.24	17.70		5.00
21.00		4.22	18.10		4.89
25.00		3.56	18.50		4.79
22.30		3.98	19.70		4.50
23.00		3.86	20.40		4.35
23.65		3.76	21.60		4.11
24.00		3.70	22.20		4.00
24.70		3.60	22.80		3.89
25.20		3.53	23.50		3.78
26.30		3.38	25.00		3.56
26.70		3.33	26.10		3.41
27.20		3.27	26.75		3.33
27.30		3.26	27.95		3.19
27.70		3.21	28.50		3.13
28.25		3.15	29.00		3.07
29.10		3.06	30.10		2.96
29.65		3.01	30.15		2.96
31.10		2.87	31.70		2.82
32.25		2.77	33.70		2.65
31.70		2.82	37.40		2.40
32.10		2.78	44.40		2.04

^aMerck Standard #8415-76^bCu-K α Radiation

<u>Wavelength (cm.⁻¹)</u>	<u>Vibrational Modes</u>
1043	11 β -OH
1135, 1117	17 α -OH
1090, 1056	21-OH
1408, 1297, 1242	1,4-diene-3-one
952, 930, 893, 852	
830, 703	

Clark lists 1655 cm.⁻¹, 1616 cm.⁻¹, and 892 cm.⁻¹ as distinctive bands for the KBr disc I.R.S. of dexamethasone.⁶

Figure 1 shows the I.R.S. of Merck Standard #8415-76. Principal bands and their assignments are tabulated below. The spectrum is in substantial agreement with published I.R.S. of dexamethasone.^{5,6,8}

<u>Wavenumber of Assignment (cm.⁻¹)</u>	<u>Vibrational Mode</u>
3400-3675	OH stretch
3020, 3050	Olefinic CH stretches
2850-3000 (several bands)	Aliphatic CH stretches
1700	C ₂₀ carbonyl
1660	C ₃ carbonyl stretch
1620, 1600	A-ring, C=C stretch
1130-1040 (several bands)	Various C-OH stretches
890	$\Delta^{1,4}$ -diene-3-ketone

2.3 Ultraviolet Absorbance

Dexamethasone exhibits a single major absorbance band at about 240 nm. in solution attributable to the Δ -1,4-3 keto A ring chromophore. The following data have been reported in the literature:

<u>Solvent</u>	<u>λ Maximum</u>	<u>$E_{1\text{ cm.}}^{1\%}$ (ϵ)</u>	<u>Reference</u>
Methanol	240 nm.	355(13,920)	(6)
Methanol	238 nm.	392(15,400)	(8)
Methanol	240 nm.	380-410	(9)

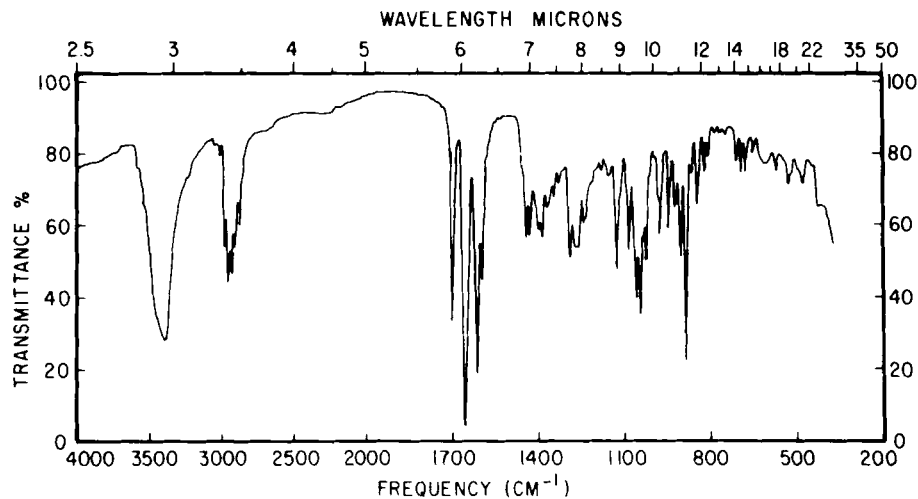


Figure 1. Infrared Spectrum of Dexamethasone; Split Mull Technique - Fluorolube mull above 1335 cm^{-1} Mineral oil mull below 1335 cm^{-1} (Merck Std. #8415-76).

Figure 2 shows an ultraviolet scan of dexamethasone (Merck Std. #8415-76) in methanol at a concentration of 0.00127%. A single absorbance maximum at 240 nm. was obtained with an $E_{1\text{ cm.}}^{1\%}$ of 393 (15,400).¹⁰

In concentrated sulfuric acid, the ultraviolet absorbance spectrum of dexamethasone undergoes a bathochromic shift characteristic of a number of steroids. Spectral data reported for dexamethasone include:

λ Max.	$E_{1\text{ cm.}}^{1\%}$	Reference
262 nm. ^a	~444	11
305 nm. ^a	~308	11
263 nm. ^b	422-455	6

^aTwo hours in concentrated H_2SO_4

^bNo conditions specified

The spectral change noted for dexamethasone in concentrated sulfuric acid is probably due to the initial protonation of the Δ -1,4-3 keto followed by a coupled chemical reaction since the spectral changes are apparently irreversible.¹²

2.4 Optical Rotation

The following specific rotations have been reported:

$$\begin{aligned}
 [\alpha]_{\text{D}}^{25^\circ} &= +75 \text{ to } 80^\circ \text{ (C = 1 in dioxane)}^9 \\
 [\alpha]_{\text{D}}^{22-24^\circ} &= 86^\circ \text{ (C = 1 in dioxane)}^8 \\
 [\alpha]_{\text{D}}^{25^\circ} &= +77.5^\circ \text{ (C = 1 in dioxane)}^1 \\
 [\alpha]_{\text{D}}^{25^\circ} &= +72 \text{ to } 80^\circ \text{ (C = 1 in dioxane)}^3
 \end{aligned}$$

2.5 Solubility

The following solubility data are given in the literature:

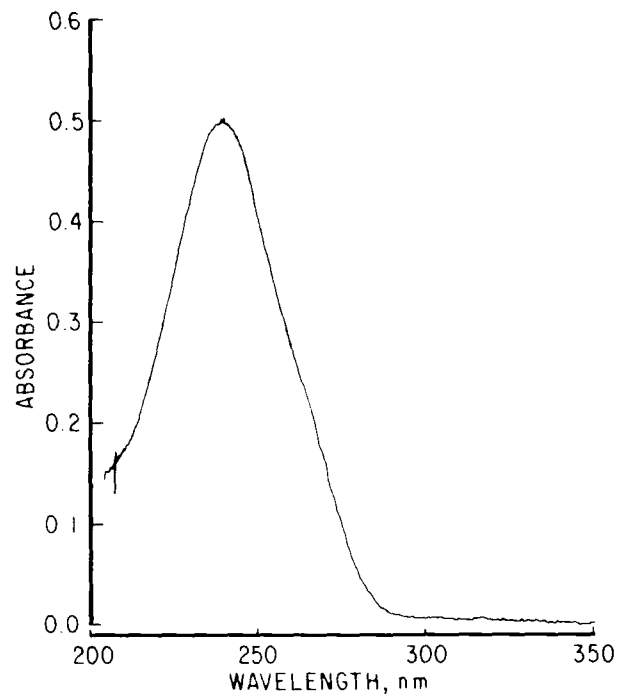


Figure 2. Ultraviolet Absorption Spectrum of Dexamethasone in Methanol at a Concentration of 0.00127% - λ_{max} 240 nm. and $E_{1\%}^{1\text{cm}} = 393$ (Merck Std. #8415-76).

<u>Solvent</u>	<u>Temperature</u>	<u>Solubility</u>	<u>Reference</u>
Distilled Water	37°C.	11.6 mg/100 ml	14
Distilled Water	25°C.	8.4 mg/100 ml	15
Distilled Water	25°C.	10.0 mg/100 ml	1
Isopropyl Myristate	37°C.	23.3 mg/100 ml	14
Light Mineral Oil	37°C.	0.01 mg/100 ml	14
Ethanol	--	1 in 42	9
Chloroform	--	1 in 165	9
Acetone	--	soluble	9
Ether	--	very slightly soluble	13
Ethyl Acetate	25°C.	4.1 mg/g	16
Pyridine	--	~10%	7

2.6 Nuclear Magnetic Resonance Spectrum (NMRS)

The 60 MHz NMRS of a 10% solution of dexamethasone in deuterated pyridine (d_5) is shown in Figure 3. Tabulated below are the proton assignments for the observed chemical shifts and coupling constants.⁷

<u>Chemical Shift ppm¹</u>	<u>J²</u>	<u>Relative # of Protons</u>	<u>Assignments</u>
7.5 ₅ /doublet	10 Hz	~1.1	C(1)-H
6.6 ₇ /crude doublet	--		11-OH ⁴
6.5 ₄ /quartet	2 Hz, 10 Hz	3.9	C(2)-H
6.4 ₃ /singlet	--		17-OH ⁴
6.3 ₅ /unresolved multiplet	--		C(4)-H
5.9 ₄ /broad singlet	--	1.1	21-OH ⁴
4.9 ₇ /AB quartet	19.5 Hz	3.1	C(21)-H ₂
4.7 ₃ /crude doublet	--		C(11)-H
1.7 ₄ /singlet	--		C(19)-H ₃
1.4 ₁ /singlet	--		C(18)-H ₃
1.1 ₃ /doublet	7 Hz	19.8	16-CH ₃
0.80-3.80 complex overlapped signals			All remain- ing protons

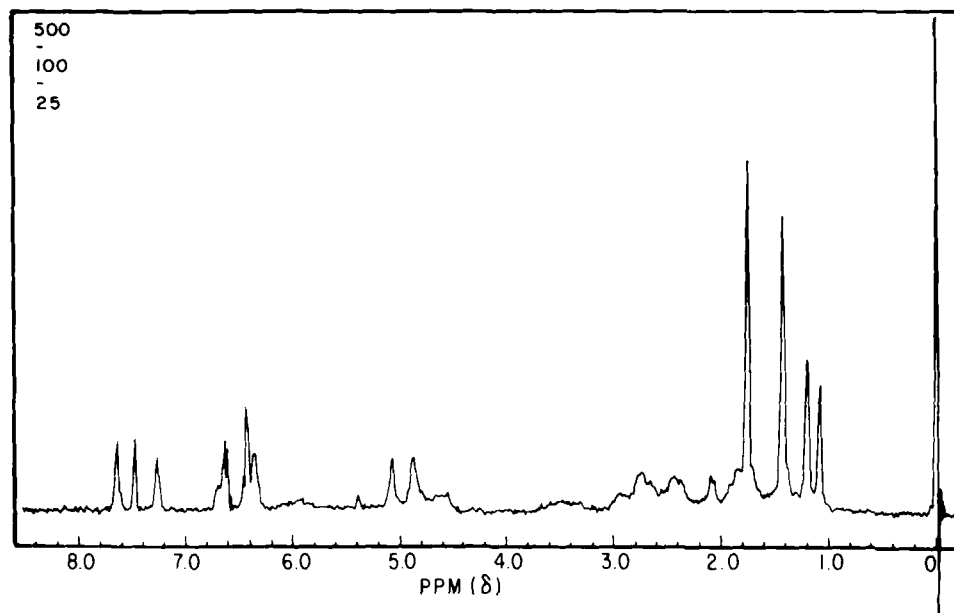


Figure 3. NMR Spectrum of Dexamethasone in Deuterated Pyridine; Operating Conditions Signal: 60 MHz Sample: Merck Std. #8415-76, 51.5 mg./0.5 cc. of C_5D_5N , Sweep Time: 250 sec. Spectrum Amplitude: 1.25×10 . Internal Standard: Tetramethylsilane

¹TMS used as internal reference

²Coupling constant

³Based on 29 protons for sum of total spectral integral

⁴Assignment confirmed via D₂O exchange

2.7 Thermal Behavior

Melting Point - The melting point of dexamethasone is reported to depend in part on the rate of heating and the degree of powder fineness.¹¹ Some of the reported values include:

<u>Melting Point</u>	<u>Reference</u>
~250°	13
~255°	9
263° - sample recrystallized from ether	17
256-258°	8
262-264° - crystals from ether	1
268-272°	1

Differential Scanning Calorimetry - The thermogram obtained on a Perkin Elmer DSC 1-B, 20°/minute for dexamethasone (Merck Std. #8415-76) exhibits the following thermal events (Figure 4):

1. Small exotherm at ~254-258°
2. Complex melting endotherm - 258° - onset temp.
- 267° - peak temp.

2.8 Mass Spectra (MS)

The low resolution MS of dexamethasone has been described in the literature.^{18,19} Important features of the spectrum include:

1. Absence of a molecular ion peak.
2. Major peak at m/e 343 (M.W. - 49) attributed to loss of water from the D-ring followed by cleavage of the C₁₈-C₂₁ bond.
3. The most intense peak of the MS occurs at m/e 122 accompanied by a major peak at m/e 121 arising from cleavage of C₆-C₇ and C₉-C₁₀ bonds and transfer of two (m/e = 122)

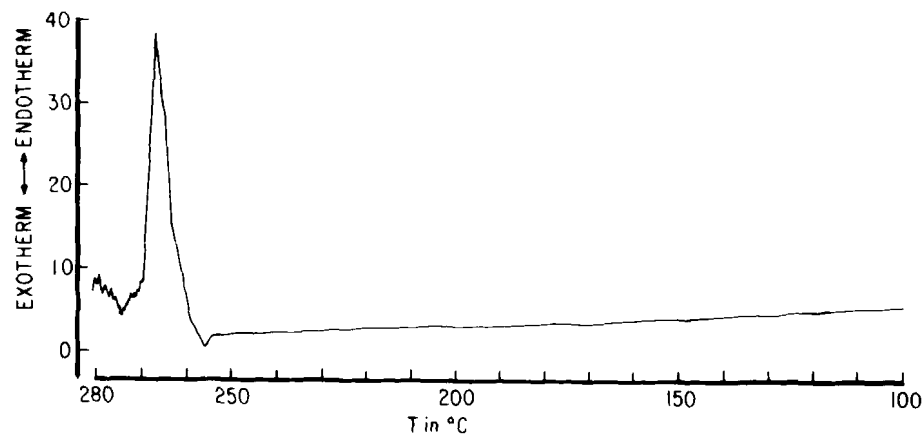
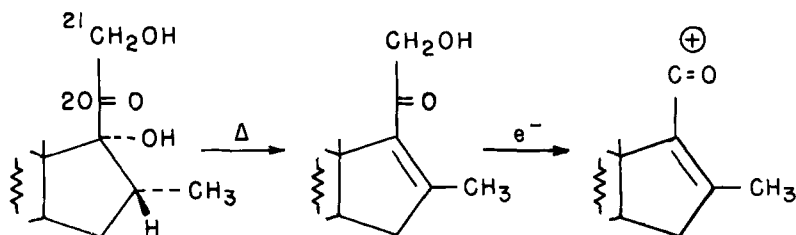


Figure 4. D.S.C. Thermogram of Dexamethasone (Merck Std. #8415-76); Perkin Elmer - D.S.C. - 1B; $20^{\circ}/\text{min.}$; sealed pan with N_2 sweep.

or one hydrogen ($m/e = 121$) to the charged fragment.

The authors^{18,19} claim that betamethasone, the 16 β -methyl isomer of dexamethasone, can be unequivocally distinguished from dexamethasone by the virtual absence of the m/e peak at 343.

Formation of the m/e 343 diagnostic fragment was attributed to a facile trans-elimination of water from dexamethasone followed by cleavage of the allylic C_{20} - C_{21} bond. It is quite conceivable that initial elimination of water occurred under thermal stress rather than under electron impact alone in the reported data.^{18,19}



Attempts to corroborate the reported literature findings with respect to the presence of m/e 343 as a major peak for dexamethasone were not successful in Merck laboratories.²⁰ Figure 5 shows the low resolution MS of dexamethasone obtained on an LKB-5000 mass spectrometer at 70 eV ionization energy. Significant differences between the spectra of dexamethasone and betamethasone were found in the relative peak height at m/e 315 and 343, as listed below (in percent of the base peaks at m/e 122).

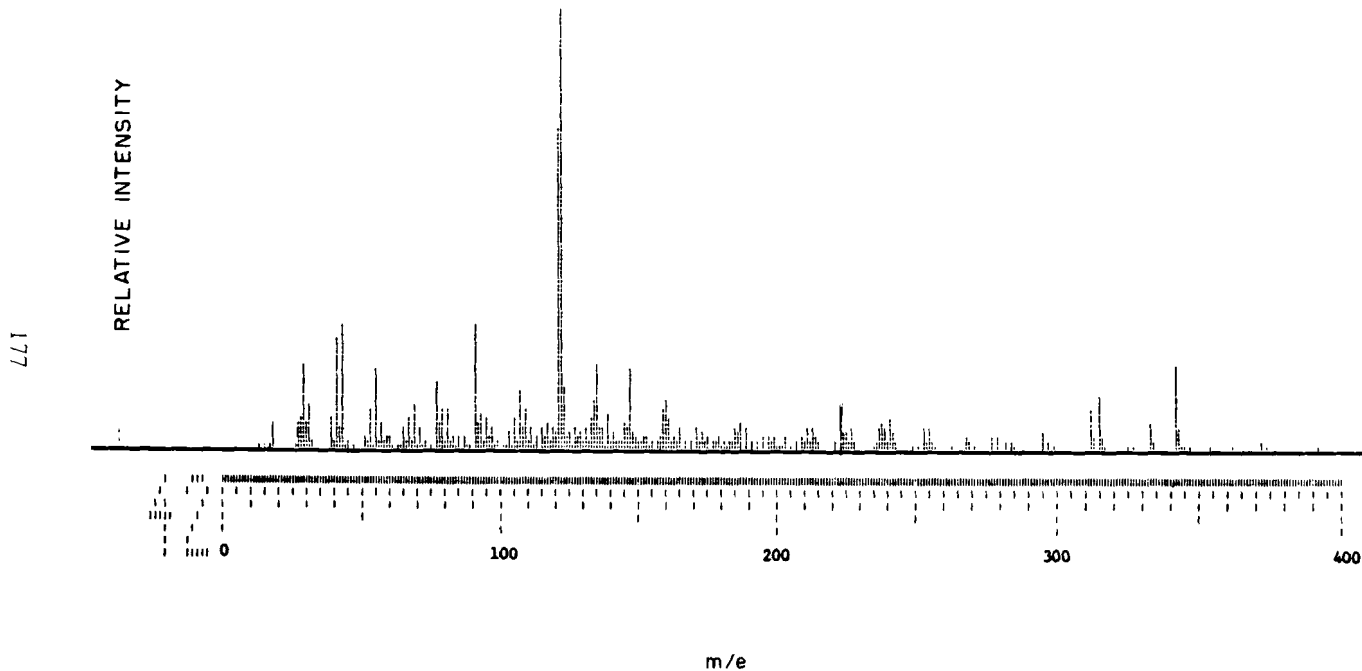
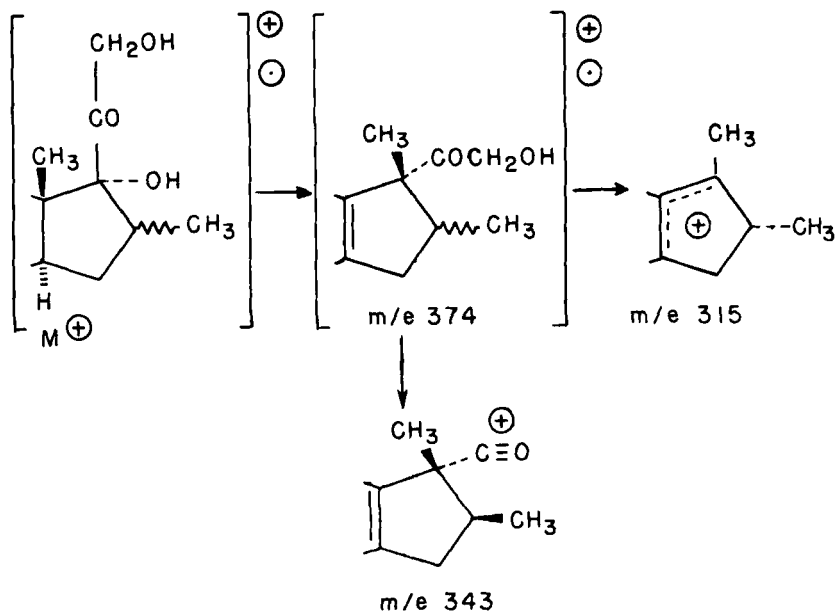


Figure 5. Mass Spectrum of Dexamethasone (Merck Std. # 8415-76)
LKB-5000 - 70 eV ionization energy.

	<u>Dexamethasone</u>	<u>Betamethasone</u>	
m/e 392	~1%	~1%	M+
m/e 374	~1%	~1%	M-H ₂ O
m/e 372	~1%	~1%	M-HF
m/e 362	~1%	~1%	M-CH ₂ O
m/e 354	~1%	~1%	M-(H ₂ O + HF)
m/e 343	5%	15%	see below
m/e 342	19%	13%	362 - HF
m/e 333	6%	3%	M-(CO-CH ₂ OH)
m/e 315	12%	3%	see below
m/e 312	9%	10%	372-(CO-CH ₂ OH + H)

A reasonable interpretation of these two fragments is based on the well known acid catalyzed water elimination from 17- α -hydroxy steroids with concomitant migration of the 18-methyl group from C-13 to C-17. In the case of dexamethasone the cis-configuration of the C-17 ketol and C-16-methyl groups in the rearranged ion m/e 374 favors the direct breakdown to m/e 315 while the trans-configuration (betamethasone) allows for a higher percentage of the intermediate ion m/e 343.



3. Synthesis

Dexamethasone can be synthesized starting from 16 α -methyl hydrocortisone acetate, an available intermediate of bile acid origin.²¹ (Figure 6). While the original synthetic scheme required a selenium oxide 1-dehydrogenation²², subsequent work established that 1-dehydrogenation could be accomplished for any number of appropriate intermediates by using microorganisms of the class Schizomycetes.²³ An entirely different synthetic scheme for dexamethasone starting from diosgenin has also been reported.²⁴

The synthesis of both tritium labeled and carbon-14 labeled dexamethasone has been given in the literature.²⁵ The following labeled material has been prepared:

1. ³H at positions 1, 2 and 4.
2. General tritium labeling.
3. ¹⁴C in the 16 α -methyl carbon.
4. ³H in the 16 β -hydrogen.

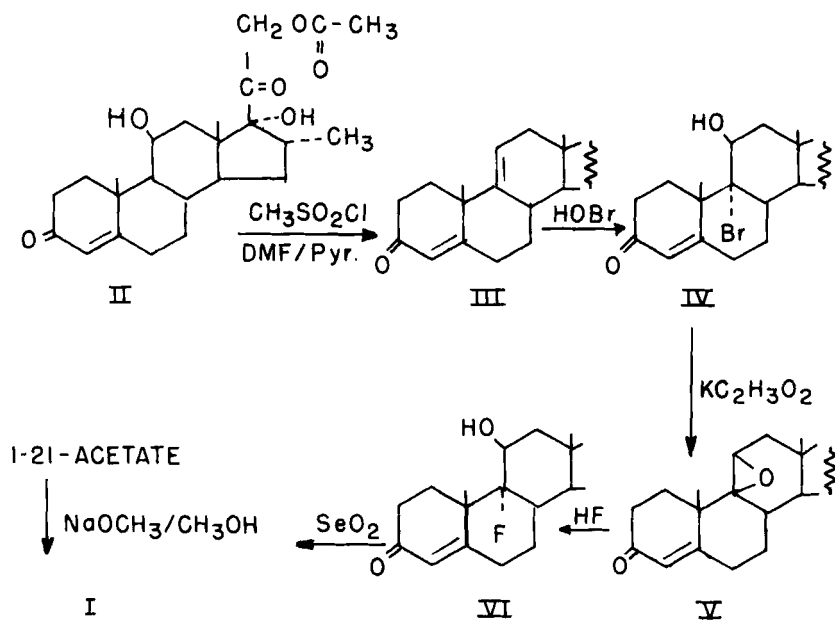
This type of labeled material provides a very sensitive handle for use as a tracer in subsequent chemical and biological studies.

4. Stability

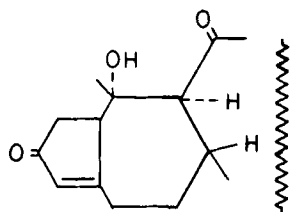
Chemical properties of the reactive moieties of dexamethasone - A consideration of the stability information reported for dexamethasone is best viewed in connection with the molecular changes reported for dexamethasone as well as structurally related adrenocorticosteroids.

A-Ring - The $\Delta^{1,4}$ -3-keto grouping of prednisone acetate can undergo photocatalyzed transformations leading to a variety of compounds whose composition depend on the conditions of the experiments.²⁶ Two such characteristic products are shown below. Further irradiation of Compound VIII can lead to a photoinduced diene-phenol rearrangement. It is reasonable to assume that dexamethasone can undergo similar photochemistry with the course of reaction duely influenced by the presence of the 9 α -fluoro group.

Figure 6.

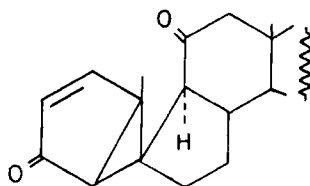


DEXAMETHASONE



VII

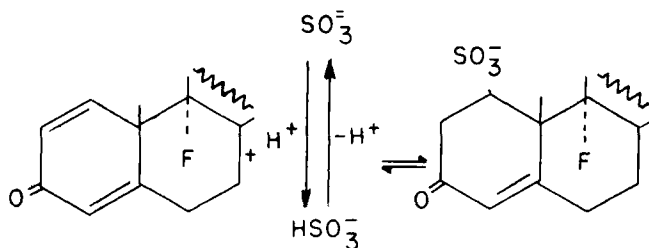
(λ max. \sim 233 nm. in ETOH)



VIII

(Lumiprednisone, λ max.
 \sim 218 nm. and 265 nm.)

Sodium bisulfite adds reversibly to the Δ^{-1} position of dexamethasone-21-phosphate sodium forming a 1-sulfonate as shown below.²⁷



1-21 PHOSPHATE SODIUM

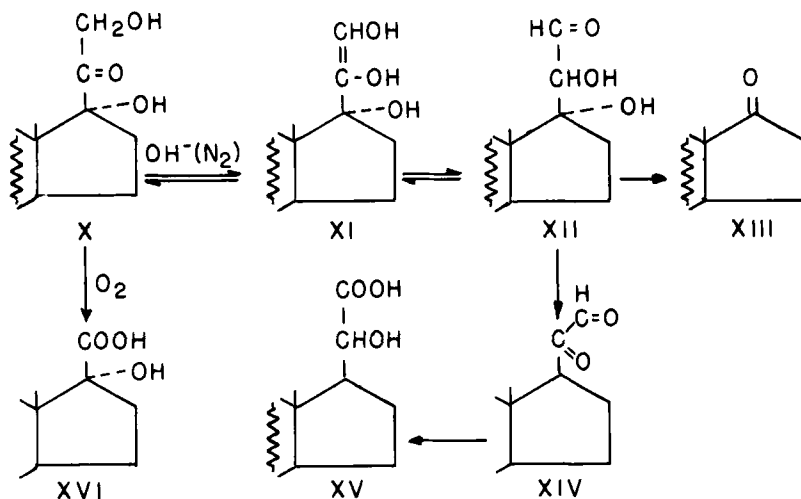
IX

From the data obtained, it is assumed that the active nucleophile is the dianionic sulfite ion. This reaction is typical of conjugate sulfite addition to α,β -unsaturated ketones. It should be inferred that other active nucleophilic agents may behave in a similar manner.

B-Ring - The 9α -fluoro aliphatic substituent would be expected to be quite stable to hydrolytic displacement. Indeed, there are no reported instances of liberation of fluoride from the 9α -fluoro adrenocorticosteroids in-vivo.²⁸

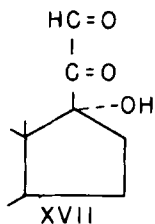
C-Ring - The 11- β -hydroxyl group of several crystalline adrenocorticosteroid esters undergoes air oxidation, catalyzed by both temperature and light, to form the corresponding 11-one compounds.²⁹ Structural requirements favoring this transformation are the formation of a non-stoichiometric solvate which undergoes facile desolvation without change in the X-ray discernible crystalline lattice structure. It was also shown that 11- β -hydroxy steroids can be air oxidized in solution under somewhat rigorous conditions. In general, the reaction requires molecular oxygen and produces water. It is accelerated by heat, greatly accelerated by free-radical initiators or by U.V. light, and is quenched by free-radical inhibitors. These authors did not report on any free C-21 alcohols.

D-Ring - The C-17 dihydroxy acetate side chain, a characteristic structural feature of all adrenocorticosteroids, is quite susceptible to both aerobic and anaerobic transformations. The anaerobic changes of the side chain are considered by Wendler as follows:³⁰

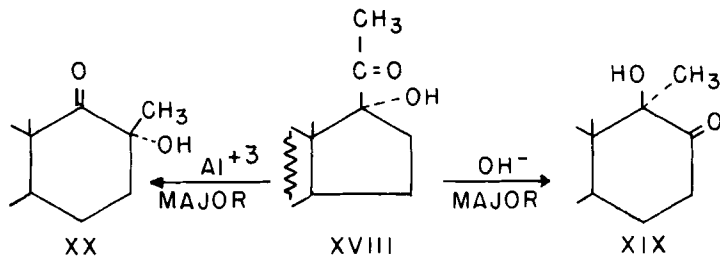


Conversion of X to the C-17 ketone (XIII) is considered as a reverse aldol reaction. Conversion of the keto-aldehyde (XIV) to the hydroxy acid (XV) is considered a benzillic acid rearrangement.

The predominant reaction, under base catalyzed aerobic conditions, appears to be oxidative cleavage of the side chain to the corresponding etianic acid (XVI). Formation of C-17 ketone is only a minor reaction product.³¹ An alternative mode of oxidative change at the C-21 position is the formation of a glyoxal (XVII) side chain which was found to be metal catalyzed.³² This glyoxal might be expected to undergo coupled chemical reactions consistent with the reactivity of that functionality.



The D-homo rearrangement reported for 17 α -hydroxy-20-keto steroids is adequately described by Wendler.³³ Possible products of this rearrangement are shown below.



While not specifically described in the literature, the D-homo rearrangement may be a possible occurrence for dexamethasone under certain reaction conditions by analogy with other steroids such as triamcinolone.³⁴

Other possible reactions involving the C-17 side chain include acylation of the C-21 hydroxyl group with available acylating agents. Prednisolone was reported to transesterify with aspirin to form prednisolone acetate in a pharmaceutical formulation matrix.³⁵ Finally, reactive reagents such as formaldehyde can attack the C-17 side chain to form the bis-methylene dioxy derivatives (BMD).³⁶ Formaldehyde is a potential impurity and/or reaction product present in such commonly used pharmaceutical adjuvants as polyethylene glycols.³⁷

Stability of Dexamethasone - The following reported stability information is entirely consistent with the potential reactivity discussed above. Dexamethasone solid is reported to be stable in air¹³ but should be protected from light.⁹ Solutions of dexamethasone lose about 50% of the C-17 α -ketol side chain within 6-8 minutes in the presence of a base catalyst.¹⁷ Excellent stability is shown by dexamethasone in a variety of marketed pharmaceutical dosage forms.² Wahba has compared the thermal stability of dexamethasone in four different tablet formulations.¹⁷

5. Methods of Analysis

5.1 Reference Standards

Both the U.S.P. and B.P. supply standard samples of dexamethasone. A highly purified sample of dexamethasone was prepared by equilibrating dexamethasone with ethyl acetate.¹⁶ Merck Standard #8415-76, which was prepared as described above, had a phase solubility analysis slope of $0.1\% \pm 0.1$ in ethyl acetate.

5.2 A-Ring Analysis (without derivatization)

Ultraviolet Absorption - Direct measurement of the U.V. absorbance at the λ maximum ~240 nm. is an official physical measurement in both the U.S.P. and B.P. In addition, U.V. absorption has been used as an analytical measurement for dexamethasone after previous separation techniques such as liquid partition and/or chromatography have been employed to isolate dexamethasone from a pharmaceutical matrix.^{17,39} Non-specific interference in the U.V.

measurements may sometimes be eliminated by a differential technique in which a strong reducing agent, such as borohydride, converts the $\Delta^{1,4}$ -3 keto chromophore to a saturated system devoid of absorbance at about 240 nm.⁴⁰ Determination of the U.V. absorbance of an untreated aliquot versus a reduced aliquot yields information on the steroid U.V. absorbance in the presence of extraneous absorbance. The feasibility of this approach was recently demonstrated for betamethasone.⁴¹ It should be noted that Δ^4 -3 keto steroids exhibit the same U.V. absorbance behavior as the $\Delta^{1,4}$ -3 keto steroids and direct measurements cannot distinguish between them. The judicious use of borohydride reduction may enable one to measure Δ^4 -3 keto impurities in the presence of $\Delta^{1,4}$ -3 keto steroids such as dexamethasone.

N.M.R. Measurements - An N.M.R. assay method for both bulk drug and formulations based on measuring the intensity of the proton signal from the C-1 proton at about 2.1 Tau for $\Delta^{1,4}$ -3 keto steroids including dexamethasone was recently described.⁴² The technique appears to be capable of measuring both Δ^1 -3 keto and $\Delta^{1,4}$ -3 keto compounds separately or together in the same sample. The relatively high levels of drug required for analysis (80-100 mg./sample) will limit application of the procedure as described without appropriate modifications to increase the assay sensitivity by at least two orders of magnitude.

Polarography - The polarographic determination of dexamethasone in tablet dosage forms in 80% ethanol, pH 4.2 McIlvaine buffer has been described.⁴³ The reduction step exhibited by $\Delta^{1,4}$ -3 keto steroid usually occurs at more anodic potentials, and is easily discernible from the reduction step exhibited by the corresponding Δ^4 -3 keto steroids.⁴⁴

A-Ring Analysis (with derivatization)

Hydrazone Formation - Isonicotinic acid hydrazide (INH) reacts with the A-ring carbonyl to produce a yellow hydrazone.³⁹ A number of substances that interfere with the INH assay are discussed in Reference 39. Recently, Taisho has recommended the use of INH·2HCl rather than INH

itself as a reagent for the determination of dexamethasone.⁴⁵

It is anticipated that other carbonyl reagents will likewise provide a basis for analytical methodology with dexamethasone. Forist and Johnson discuss a number of these.⁴⁶

The integrity of the A-ring of steroids is generally considered to be reliably determined by either U.V. or INH assay.^{39,47} In view of the reported products of A-ring photolysis of prednisone having α, β unsaturated ketone structures (see Section 4), which may interfere in the measurement of the unaltered A-ring steroid, caution in using these measurements should be exercised. It is interesting to note that data presented in Reference 47 suggest that both direct U.V. and/or INH assay gave higher results for photolyzed solutions of prednisone ($\Delta^1, 4-3$ keto steroid) than did a quantitative paper chromatographic assay of the same solutions.

5.3 B-Ring Analysis

There are no literature reports that deal specifically with ascertaining the integrity of the 9α -fluoro substituent in dexamethasone.

5.4 C-Ring Analysis

While there are no specific reports concerning dexamethasone, a number of procedures based on the reactivity of the oxygen function at C-11 are discussed by Forist and Johnson for related steroids.⁴⁶

5.5 D-Ring Analysis

The α -ketol group ($\text{CH}_2\text{OH}-\text{CO}$) possesses reducing properties and its reactivity towards blue tetrazolium (B.T.) is utilized extensively as an assay procedure for dexamethasone.³⁹ Both the U.S.P. and B.P. employ the B.T. assay for dexamethasone. However, the U.S.P. utilizes a prior thin layer chromatographic separation which increases the selectivity and specificity of the final B.T. assay measurement for intact dexamethasone. The presence of

easily oxidized excipients can cause interference in the B.T. assay procedure. A tabulation of a number of such interferences is given in Reference 39. Both positive and negative interferences are possible. The former are due to agents which are oxidized under the conditions of the B.T. reaction. The latter are agents which can alter the pH of the alkaline B.T. reaction mixture thereby decreasing the extent of color formation. Effective elimination of interferences can often be achieved by selective extraction and chromatographic procedures. An automated B.T. assay has been described for dexamethasone tablets.⁴⁸

The 17,21-dihydroxy-20-keto group of dexamethasone reacts with phenylhydrazine-sulfuric acid (Porter-Silber) reagent to form a yellow chromogen suitable for assay purposes.³⁹ As in the case of the B.T. assay, a number of specific color interferences, which can arise from the reaction of the reagent with excipients, may be eliminated by selective extraction and chromatographic procedures.

A number of other assay approaches based on the reactivity of the intact C-17 side chain described for related steroids are presumably applicable to dexamethasone; These include: a) 2,4-dinitrophenylhydrazine reagent⁴⁹ - this reagent was found to be approximately twice as sensitive as B.T. assay for prednisone but is not quite as selective as B.T. reagent for intact prednisone in the presence of degradation products; b) formaldehyde estimation following periodate or bismuthate oxidation.⁴⁶

The use of any of the assay procedures described above as an absolute index of dexamethasone integrity in a pharmaceutical matrix should not be assumed without independent verification utilizing high resolution mass transport procedures such as thin layer chromatography, because of the complex nature of the chemical changes possible at the C-17 side chain. For example, a TLC separation of a sample mixture followed by a demonstration of the absence of color response to a particular reagent of all foreign spots, save for intact dexamethasone, would lend assurance as to the specificity of that reagent.

5.6 Infrared Analysis

Bellomonte⁵⁰ described a solid state IR assay procedure (KBr mull) for measuring dexamethasone in the presence of triamcinolone utilizing as analytical wavelengths 915 cm^{-1} and 1140 cm^{-1} .

5.7 Fluorescence Analysis

Unlike Δ^4 -3 keto steroids, the $\Delta^{1,4}$ -3 keto compounds do not exhibit significant sulfuric acid induced fluorescence.⁵¹ Recently, a fluorescent assay for corticosteroids was described based on making a fluorescent D-ring derivative.⁶⁶

5.8 Mass Transport Techniques

Assay procedures described below depend on the selective migration of either the intact molecule or a recognizable derivative of the intact molecule between phases followed by a specific functional group or non-specific quantitative measurement for the molecule in the appropriate phase.

Phase Solubility Analysis - See Section 5.1 for discussion of a phase solubility system for dexamethasone.

Liquid-Liquid Extraction - Separation of intact adrenocorticosteroids from acidic decomposition products has been accomplished by partitioning a sample between a neutral or slightly alkaline aqueous phase and chloroform.^{31,39} A very useful discussion of the interpretation of differences noted for steroid content of samples obtained by B.T., Porter-Silber, INH and U.V. assay is given in Reference 39.

Column Chromatography - The use of high pressure liquid chromatography as a convenient stability indicating assay procedure for the analysis of corticosteroid creams and ointments was recently demonstrated.⁵² The authors show a sample chromatogram obtained for dexamethasone using a column of β, β^1 -oxydipropionitrile on Zipax and 1% ethanol in hexane as a mobile phase at 500 p.s.i. (elution time ~11 minutes). A generalized system for the prediction of

of elution curves for corticosteroids based on partition coefficients for a hexane-chloroform-dioxane-water (90-10-40-5) solvent system on diatomaceous earth was also described.⁵³ The authors give data for betamethasone. A column partition system has been described by Graham which can effectively trap the corticosteroid in an acetonitrile layer on a diatomaceous earth column while interferences are removed by washing with heptane.⁵⁴ Acetonitrile and corticosteroids are then removed from the column with chloroform. The method may be modified readily for the removal of acidic, basic, and/or water soluble interferences. Data are given for both formulated and unformulated dexamethasone.

Paper Chromatography - Johnson and Fowler give R_f data for dexamethasone and related steroids utilizing the following system:⁵⁵

Stationary Phase: Whatman No. 1 impregnated with
40% V/V formamide in methanol.
Mobile Phase: Saturated solution of formamide in
chloroform.
Mode of Operation: Descending development for 35 cm.
in a saturated tank.
Detection: Diphenyl styryl phenyl tetrazolium +
heat = violet spots for steroids.

<u>Compound</u>	<u>R_f</u>
Betamethasone	.16
Cortisone	.62
Dexamethasone	.21
Hydrocortisone	.26
Prednisolone	.15
Prednisone	.55

Thin Layer Chromatography (TLC) - U.S.P. XVIII utilizes TLC as both a qualitative and quantitative assay procedure for dexamethasone.¹³ N.F. XIII employs TLC as an identity test for dexamethasone in official dosage forms.⁵⁶ Wahba has demonstrated the utility of quantitative TLC as a stability indicating approach to the estimation of dexamethasone in aged samples of experimental tablet formulations.¹⁷ There is little doubt that TLC offers the analyst

an excellent highly selective method, usually without requiring any derivatization, for assessing the integrity of dexamethasone in common pharmaceutical matrices. Thin layer chromatography can often function as a primary or referral assay procedure to corroborate the validity of a non-chromatographic assay procedure. Some of the reported TLC data for dexamethasone, in addition to the information in the U.S.P. and N.F., are given in Table II. Other TLC systems for dexamethasone are discussed in References 17, 59 and 60. Of special interest is a report which describes the separation of dexamethasone from betamethasone.⁶¹

Gas Chromatography - Steroids possessing the C-17 dihydroxyacetone side chain usually undergo molecular alteration after application to GLC columns to yield as a major product the corresponding 17-ketosteroid.⁶² Approaches to attain quantitative conversion to a suitable non-labile derivative of steroids related to dexamethasone have been recently described by Baillie and co-workers.⁶³ One of the most stable derivatives suitable for gas chromatography is the 20-O-methyloxime-17,21-trimethylsilyl ether of the adrenocorticosteroid.

6. Metabolism and Pharmacokinetics

Normal human subjects given 1.6 mcg. of 1,2,4-³H dexamethasone orally excreted about 15% of the total radioactivity in the urine within four hours after administration.⁶⁴ Approximately 50% of the excreted radioactivity was in conjugated form, presumably a glucuronide and 50% of the excreted radioactivity was in non-conjugated form. The urinary levels of total and conjugated dexamethasone in patients on chronic diphenylhydantoin therapy were significantly increased compared to the levels shown by normal human subjects.

In another study, the mean recovery of urinary radioactivity after four hours and 24 hours was 16% and 64%, respectively for doses of 0.5 to 1.5 mg. of labeled dexamethasone administered intravenously as a solution in saline to human subjects.⁶⁰ The major pathway of dexamethasone metabolism, following I.V. administration, appears to involve formation of polar unconjugated derivatives. A

TABLE II

Silica Gel G

Solvent: ^a	A	B	C	D	E	F	G	H	I	J
Rf: ^b	1.00 ^c	.72 ^d	.51 ^d	1.40 ^d	.60 ^d	.85 ^d	.35 ^d	.52 ^d	.50 ^d	.67 ^d
Reference:	57	58	58	58	58	58	58	58	58	58

^a A = Methylene Chloride:dioxane:water (100:50:50) - lower layer;

B = Chloroform-ethanol (9:1);

C = Chloroform-90% methanol (9:1);

D = Cyclohexane-ethyl acetate (1:1);

E = Chloroform-acetone (9:1);

F = Chloroform-acetone (4:1);

G = Cyclohexane-chloroform-acetic acid (7:2:1);

H = Methylene chloride-acetone (4:1);

I = Chloroform-acetic acid (9:1);

J = Methylene chloride-acetic acid (9:1).

^b Dexamethasone separated from other related steroids. Reference 58 also includes TLC data for dexamethasone on aluminum oxide and magnesium silicate stationary phases as well as useful information on detection system.^c Relative to hydrocortisone = 1.00.^d Relative to cortisone = 1.00.

DEXAMETHASONE

$t_{1/2}$ of 252 minutes was noted for the elimination of intact dexamethasone from plasma.

It is anticipated that development of appropriate sensitive assays for dexamethasone will enable studies in human subjects to be carried out using conventional pharmaceutical dosage forms, made with "cold" dexamethasone, to yield additional information on the oral bioavailability of dexamethasone. Current analytical methodology for dexamethasone is not sufficiently sensitive to assay at the nanogram per ml. level, which represents expected plasma concentrations following oral administration of therapeutic doses of dexamethasone. Techniques such as radioimmunoassay⁶⁵ are likely to provide the assay sensitivity and selectivity required for these studies in the presence of the normal steroid background in biological fluids.

A procedure for the detection and identification of dexamethasone in horse urine has been described and has a sensitivity limit of 2 mcg. per ml. of urine taken for analysis.⁵⁹

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DIOCTYL SODIUM SULFOSUCCINATE

Sut Ahuja and Jerold Cohen

Reviewed by J. Kazan and T. E. Ricketts

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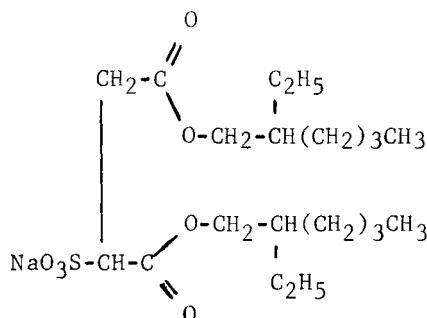
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1. DESCRIPTION

1.1 Name, Formula, Molecular Weight, Elemental Composition

Chemically, dioctyl sodium sulfosuccinate is sulfosuccinic acid bis(2-ethylhexyl) ester S-sodium salt or sodium 1, 4-bis(2-ethylhexyl) sulfosuccinate or bis(2-ethylhexyl) sodium sulfosuccinate. It is also known as sodium dioctyl sulfosuccinate; DSS; Aerosol OT; Alphasol OT; Colace; Complemix; Coprol; Dioctylal; Dioctyl-Medo Forte; Diotilan; Diovac; Disonate; Doxinate; Doxol; Dulsvivac; Molatoc; Molofac; Nevax; Norval; Regutol; Softili; Solusol; Sulfimel DOS; Vatsol OT; Velmol and Waxsol (1). It has a molecular weight of 444.57 ($C_{20}H_{37}O_7SNa$).

DIOCTYL SODIUM SULFOSUCCINATE



The theoretical elemental composition of dioctyl sulfosuccinate is as follows:

Carbon	54.03%
Hydrogen	8.39%
Oxygen	25.19%
Sulfur	7.21%
Sodium	5.17%

1.2 Appearance, Color, Odor

Dioctyl sodium sulfosuccinate is grayish to white, wax-like, plastic solid, having a characteristic odor suggestive of octyl alcohol.

2. PHYSICAL PROPERTIES

2.1 Infrared Spectrophotometry

The infrared spectrum (2) of a 5% chloroform solution of dioctyl sodium sulfosuccinate recorded with a Perkin-Elmer Infrared Spectrophotometer, Model 225 is shown in Figure 1. Band assignments for the spectrum in 5% chloroform solution are listed in Table I.

2.2 Nuclear Magnetic Resonance

The NMR spectrum (3) of dioctyl sodium sulfosuccinate (Figure 2) was recorded in DMSO-d₆ on a varian A60 60MHz NMR spectrometer. Structural assignments for the NMR signals are tabulated in Table II.

2.3 Mass Spectrometry

Reliable mass spectral data (4) could not be obtained for dioctyl sodium sulfosuccinate as the spectrum changes with temperature, indicating thermal degradation.

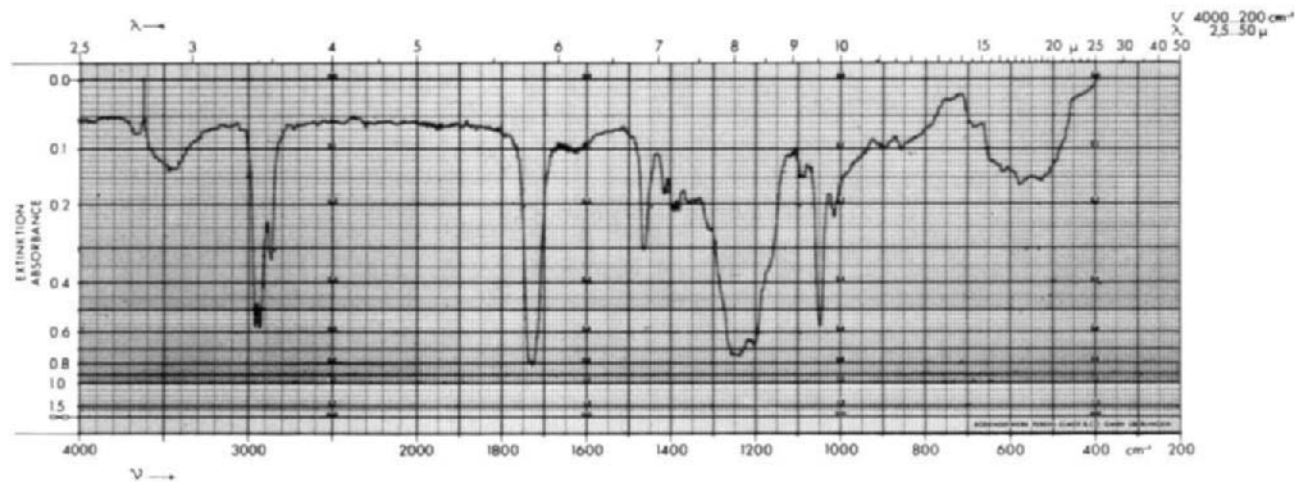


FIGURE I. Infrared Spectrum of a 5% chloroform solution of Dioctyl Sodium Sulfosuccinate

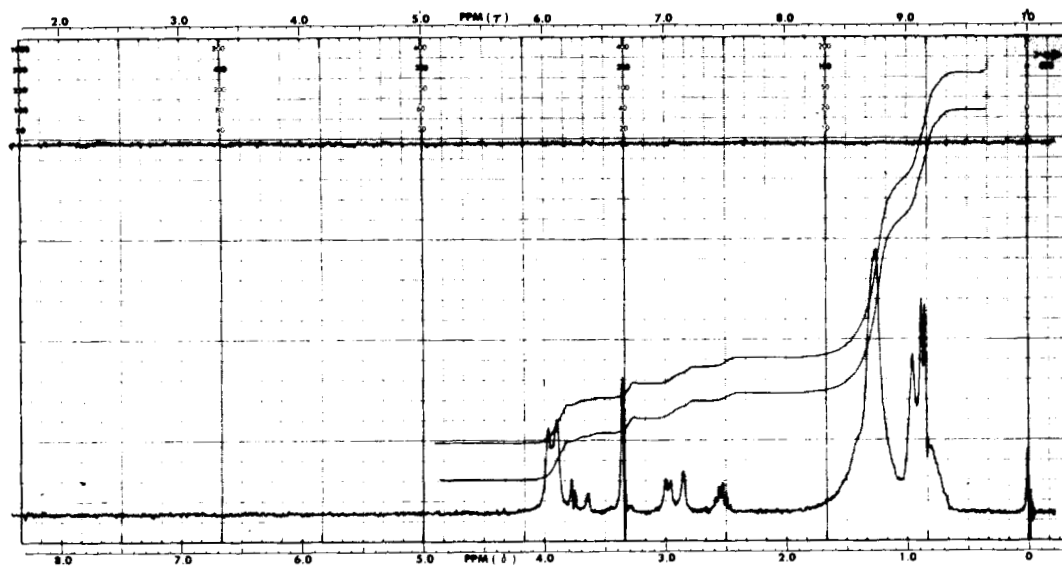


FIGURE 2. Nuclear Magnetic Resonance Spectrum in DMSO-d₆ of Dioctyl Sodium Sulfosuccinate

TABLE I
INFRARED BAND ASSIGNMENTS FOR
DIOCTYL SODIUM SULFOSUCCINATE
IN 5% CHLOROFORM SOLUTION

Wavenumber (cm ⁻¹)	Assignment
2960, 2935, 2865	aliphatic CH ₂ ; CH ₃
1728	C = O
1465	aliphatic CH ₂ ; CH ₃
1391, 1381	CH ₃
1245	asymmetric C-O-C (ester)
1200	asymmetric SO ₃ [⊖]
1048	symmetric C-O-C (position) corresponds to C-O-CH ₂ and/or symmetric SO ₃ [⊖]

2.4 Differential Thermal Analysis

The thermal analysis (5) of dioctyl sodium sulfosuccinate was conducted on a Du Pont Model 900 thermal analyzer in a nitrogen atmosphere. At heating rates of 10°C/minute and 3°C/minute, the thermogram (Figure 3) shows no endotherm, but an irregular curve after 120°C. No transition was observed except foaming of the sample.

2.5 Thermogravimetric Analysis

A sample of dioctyl sodium sulfosuccinate (Figure 4) loses weight (5) continuously above 35°C: 3.4% between 35°C and 152°C, approximately 0.5% between 152°C and 220°C. Rapid weight loss occurs above 220°C, which amounts to approximately 5.8% loss up to 248°C. A residue of approximately 15% is left at 360°C.

2.6 Differential Scanning Calorimetry

A thermogram run at 10°C/minute shows two broad endotherms (5) between 119°C and 194°C (Figure 5).

2.7 Solubility

Dioctyl sodium sulfosuccinate is soluble in the following solvents: carbon tetrachloride, petroleum ether, naphtha, xylene, dibutyl phthalate, liquid petrolatum,

SAMPLE: <u>Budget Sodium Sulfate 400</u> <u>89 3176</u> ANAL: <u>71339</u> ORIGIN: <u>Nickolan</u>	SIZE <u>6.2000 g 1/2 100.275</u> REF: <u>Calom. Bands</u> PROGRAM MODE <u>Heat</u> RATE <u>10</u> Δ START <u>RT</u> °C	ATM. <u>16</u> <u>1 SEC</u> <u>T</u> Δ T SCALE <u>20</u> Δ <u>0.4</u> Δ SETTING	RUN NO. <u>9170</u> DATE <u>1/1/70</u> OPERATOR <u>MF 31/81</u>
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EXO
 ↑
 Δ T
 ↓
 ENDO

T. °C (CORRECTED FOR CHROMEL ALUMEL THERMOCOUPLES)

SAMPLE: Acetyl Sulfonamide
 BP 5176
 Anal. 9.12.89
 SIZE 11.6 mg. Nicolson

X-AXIS
 TEMP. SCALE 47 °C
 SHIFT — inch
 TIME SCALE (ALT.) —

Y-AXIS
 SCALE 0.4 mg.
 (SCALE SETTING X 2)
 SUPPRESSION 28.37 mg.

RUN NO. 27 DATE 6/6/72
OPERATOR JWF 23/62
HEATING RATE 10 °C/min.
ATM. 46
TIME CONSTANT 1 sec.

TGA
 ~90% Suppression
 0 Suppression
 no suppression scale change

WEIGHT
 22.37
 22.17
 21.97
 21.77
 21.57
 21.37
 21.17
 20.97
 20.77
 20.57
 20.37
 20.17

TEMP. °C (CORRECTED FOR CHROMEL ALUMEL THERMOCOUPLES)
 0 50 100 150 200 250 300 350 400 450 500

205

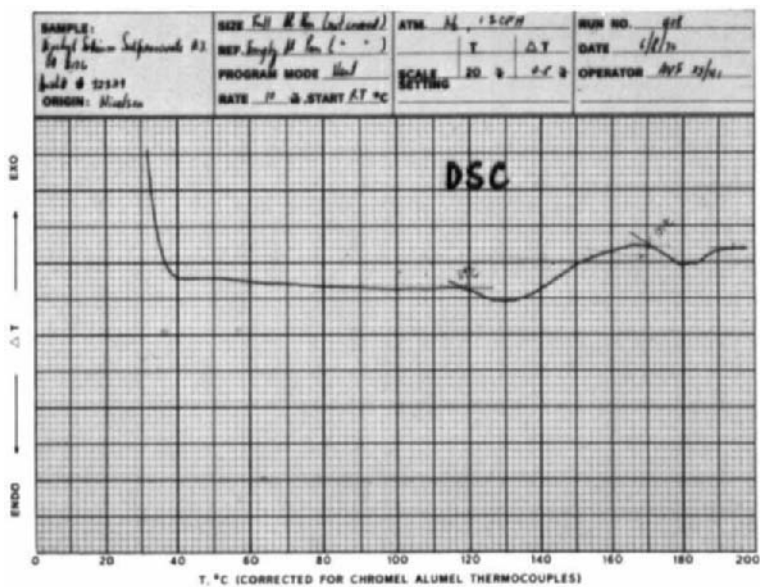


FIGURE 5. Differential Scanning Calorimetry thermogram of Diethyl Sodium Sulfosuccinate

DIOCTYL SODIUM SULFOSUCCINATE

TABLE II

NMR SPECTRAL ASSIGNMENTS FOR DIOCTYL SODIUM SULFOSUCCINATE

(ppm)	Multiplicity	Number of Protons	Assignment
0.6-1.9	undetermined	30	$\left[\begin{array}{c} \text{CH}_2\text{-CH}_3 \\ \\ \text{-C-(CH}_2\text{)}_3\text{-CH}_3 \\ \text{H} \end{array} \right] \times 2$
2.8-3.1	undetermined	2	$\begin{array}{c} \text{CH}_2\text{-C(=O)} \\ \\ \text{S-CH-C(=O)} \\ \quad \quad \quad \text{O} \end{array}$
3.35	singlet	1.8	H_2O
3.6-4.1	undetermined	5	$\begin{array}{c} \text{CH}_2\text{-C(=O)-O-CH}_2 \\ \\ \text{S-CH-C(=O)-O-CH}_2 \\ \quad \quad \quad \text{O} \end{array}$

glycerol, pine oil, oleic acid, acetone, kerosene, alcohol, benzene, diacetone alcohol, methanol, ethanol, isopropanol, **sec. butanol, methyl acetate, ethyl acetate, amyl alcohol,** furfuryl alcohol, tetrahydrofurfuryl alcohol, polyethylene glycol, corn oil, and vegetable oils (1). Table III presents the solubility of dioctyl sodium sulfosuccinate in selected solvents. The solubility figures shown do not indicate the limits of solubility, since dioctyl sodium sulfosuccinate appears to be very highly soluble in most organic solvents (6).

TABLE III
SOLUBILITY OF DIOCTYL SODIUM SULFOSUCCINATE (DSS) IN SOME ORGANIC SOLVENTS*

Method of Solution		Specific Gravity		DSS in Solution		Viscosity of Solution (Poises)
		g/ml at 25°C		Percent		
		Dissolving Liquid	Resulting Solution	g/100 ml	by Weight	
Dissolved at	Carbon Tetrachloride	1.55	1.31	73.8	56.4	1.65
Room Temp.	Petroleum Ether	0.688	0.950	70.1	75.0	0.65
Heated then	Solvent Naphtha	0.864	0.701	70.5	69.8	0.65
Cooled	Dibutyl Phthalate	1.03	1.07	70.7	66.1	8.84
	Paraffin Oil	0.881	1.00	69.5	69.5	12.9

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DIOCTYL SODIUM SULFOSUCCINATE

Dioctyl sodium sulfosuccinate dissolves slowly in cold water, but its solubility in water increases with an increase in temperature, as indicated in Table IV.

Table V presents the concentration of electrolyte solution in which 1% of dioctyl sodium sulfosuccinate is soluble at a temperature of 25°C.

TABLE IV

SOLUBILITY OF DSS IN WATER*

<u>Temperature °C</u>	<u>Grams/100 ml of Water</u>
25	1.5
30	1.8
40	2.3
50	3.0
60	4.0
70	5.5

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TABLE V

SOLUBILITY OF 1% DSS IN ELECTROLYTE SOLUTIONS*

Concentration of Electrolytes, %						Appearance of Solution
NaCl	NH ₄ Cl	(NH ₄) ₂ HPO ₄	NaNO ₃	Na ₂ SO ₄		
0.5	0.5	2.0**	0.5	1.0	Clear	
3.0	2.0	3.0	1.0	3.0	Turbid	

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** Slightly turbid

2.8 Solubilization

Table VI presents the amount of solubilizing agent (solvent) necessary to produce 10% and 25% clear solutions (w/w) in the solvent and water at room temperature. The values represent the amount of DSS and solvent to be added.

TABLE VI
SOLVENTS CAPABLE OF SOLUBILIZING
AQUEOUS SOLUTIONS OF DSS*

<u>Solvent</u>	<u>Solvent Required For 10% Solution, % By Weight</u>	<u>Solvent Required For 25% Solution, % By Weight</u>
Acetone	17.5	-
Amyl alcohol (clear thick gel)	5.9	-
Ethyl alcohol	15.0	12.0
2-Butanol	11.5	-
Butyl carbitol**	12.0	15.0
Butyl cellusolve**	12.0	15.0
Diacetone alcohol	18.0	15.0
Diethylene glycol	15.0	12.0
Ethyl lactate	10.0	-
Furfuryl alcohol	7.4	-
Isopropyl alcohol	17.5	15.0
Methanol	12.5	13.0
Methyl acetate	15.0	15.0
Pine oil	Not satisfactory	5.0
Synosol** solvent	17.5	15.0
Tetrahydro furfuryl alcohol	9.9	-

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Diocetyl sodium sulfosuccinate can act as a solubilizing agent and can also be acted upon by other solubilizing agents (7). Diocetyl sodium sulfosuccinate can bring into solution, in water, materials which are normally in-

soluble or only sparingly soluble (e.g. organic acids, dyes, proteins, germicides, gums, etc.). Good solubilization can be achieved by ensuring that DSS is uniformly distributed throughout the bulk of particles to be dissolved. The proportion of DSS used should preferably be between 0.1-1.0% of the material to be dissolved (6). The exact mechanism of solubilization is not known. However, it apparently involves micellar activity (8-11).

2.9 Effect On Surface Tension of Liquids

DSS lowers the surface tension of liquids e.g. water and various electrolyte solutions. The effect of DSS on surface tension may be measured by a variety of methods; however, the Pendant Drop Method (12) seems to be most satisfactory for measuring changes in surface tension. The effect of concentration of DSS on surface tension as determined by Pendant Drop Method is shown in Table VII.

TABLE VII

SURFACE TENSION OF DIOCTYL SODIUM SULFOSUCCINATE
BY PENDANT DROP METHOD
TEMPERATURE, 25°C. SURFACE AGE, 5 SECONDS*

Concentration DSS % Solids	Surface Tension (dynes/cm.)				
	Water	0.25% NaCl	0.5% NaCl	1.0% Na ₂ SO ₄	2.0% Na ₂ SO ₄
0	72.0	-	-	72.5	72.8
0.001	62.8	52.4	40.1	42.0	41.5
0.02	38.9	26.3	25.3	25.9	26.0
0.1	28.7	24.9	24.8	24.6	25.2
0.25	28.5	24.3	25.2	25.6	25.4
0.5	27.5	25.3	25.5	25.2	25.2
1.0	26.0	Cloudy	Cloudy	Cloudy	Cloudy
2.0	Cloudy	Cloudy	Cloudy	Cloudy	Cloudy

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2.10 Crystal Properties

X-ray diffraction analysis (5) of dioctyl sodium sulfosuccinate was conducted on a Philips Norelco Diffractometer Unit (scintillation counter), using the following conditions:

Radiation:	Cu K α , 35 KV. 15 ma
Filter:	Nickel
Scanning Rate:	1/2° (20)/minute
Chart Speed:	1/2 inch/minute
Divergence Slit:	1°
Receiving Slit:	0.006 inches
Scatter Slit:	1°
Time Constant:	2 seconds
Counts, Full Scale:	1000 cps

The results of X-ray diffraction analysis are in Table VIII (Figure 6).

TABLE VIII

X-RAY DIFFRACTION ANALYSIS

<u>Bragg Angles</u> <u>(degree 2 θ)</u>	<u>Relative</u> <u>Intensity</u>
4.29	100

3. SYNTHESIS

Several patents have been issued covering the preparation of DSS (13, 14). The synthesis of dioctyl sodium sulfosuccinate is generally accomplished by the treatment of maleic acid anhydride with 2 - ethylhexanol to produce dioctyl maleate which is then reacted with sodium bisulfite under conditions conducive to saturation of the olefinic bond, with simultaneous rearrangement of the bisulfite to the sulfonate structure (15).

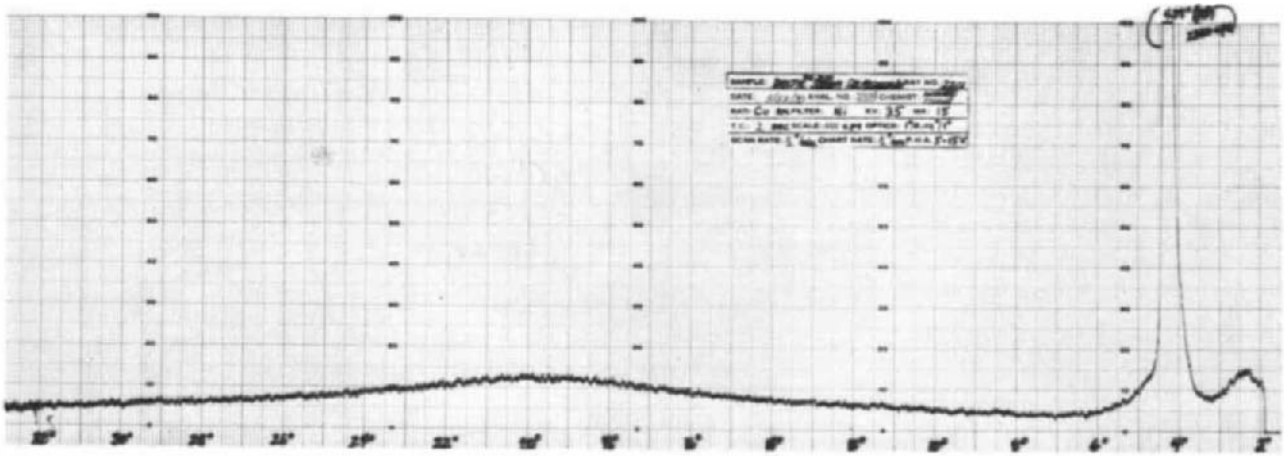
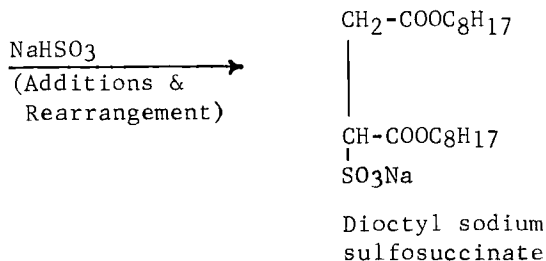
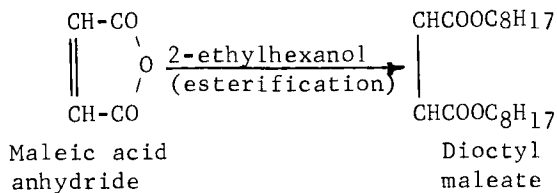


FIGURE 6. X-Ray Diffraction Analysis of Dioctyl Sodium Sulfosuccinate



Calcium, potassium, magnesium, aluminum hydroxide, and ammonium salts of dioctyl sulfosuccinate have been prepared (16-20) but have not found applications comparable to DSS.

4. STABILITY

Dioctyl sodium sulfosuccinate is a fairly stable compound; after 12 weeks of storage at 60°C, no decomposition was observed by TLC (21). Since dioctyl sulfosuccinate is an ester, it can be hydrolyzed in the presence of either strongly acidic or strongly alkaline media. Extensive information on the stability of DSS as a wetting agent is available (6). Figure 7 shows the stability of DSS at various pH values. The stability was measured by Draves Test which measures the wetting power i.e. rate of spreading and imbibition.

DSS may be used to improve the physical stability of other materials such as:

- a) a stabilizer for cocoa butter in beverages (22)
- b) improving dye uptake and thermal stability of polyacrylonitrile yarn (23)
- c) stabilization and coagulation of Ag Br sols (24)
- d) improving the stability of poly (vinyl acetate) emulsions to freezing and thawing (25)

DIOCTYL SODIUM SULFOSUCCINATE

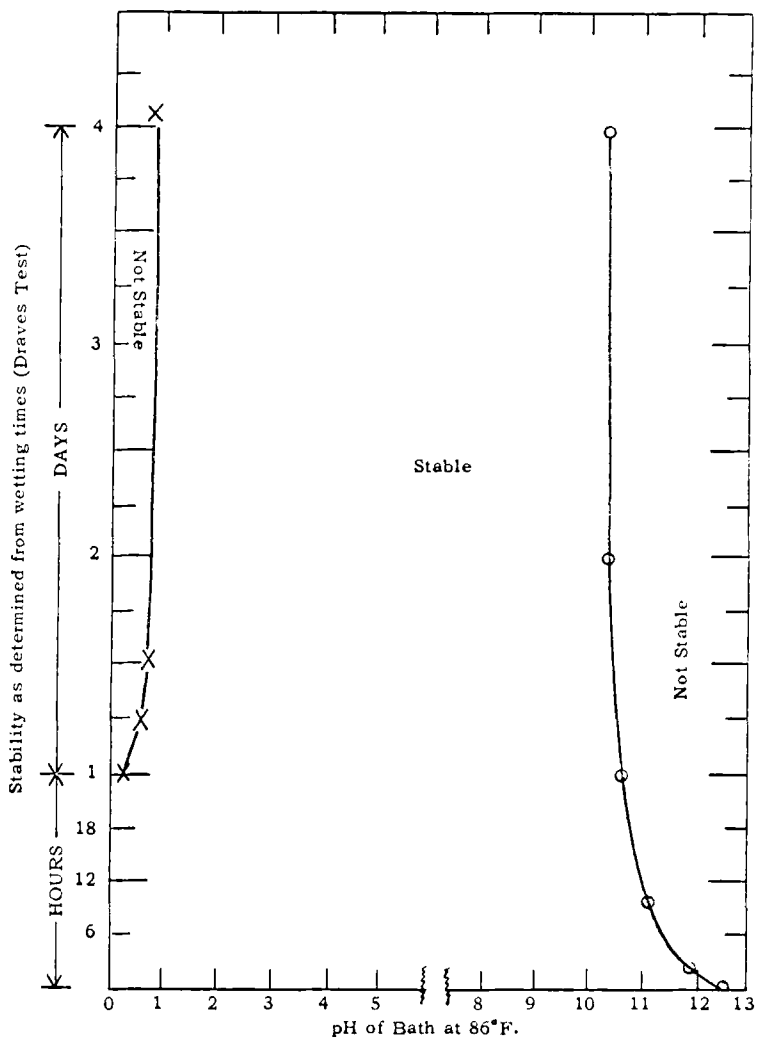


Figure 7. Stability of Solutions of Dioctyl Sodium Sulfosuccinate (0.025% in solution at various pH values) *

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5. METABOLISM

In human beings, dioctyl sodium sulfosuccinate is not absorbed or altered in the intestinal tract (6). Experiments with mice appear to confirm this. Furthermore, DSS does not aid in the absorption of other materials in solution such as insulin, histamine, diamino-diphenyl sulfone (26). However, it enhances the penetration of sulfanilamide, sulfathiazole, sulfadiazine and sulfapyridine into the cornea, sclera and aqueous humor of human beings (27).

6. METHODS OF ANALYSIS

6.1 Titrimetric Analysis

Dioctyl sodium sulfosuccinate can be assayed by titration against standard tebrabutylammonium iodide with bromphenol blue as an indicator, in the presence of chloroform (28).

DSS can also be determined by titration against cetylalkonium chloride solution with bromophenol blue as an indicator, in the presence of chloroform (29).

6.2 Colorimetric Analysis

DSS forms a complex with methyl green which is soluble in benzene and can be determined spectrophotometrically at 615 nm (6).

Another method of determination of DSS is based on formation of the dioctyl sulfosuccinate salt of methylene blue, extraction of the salt into chloroform, and spectrophotometric determination of the extracted salt at 650 nm (30).

Determination of DSS by reaction with basic fuchsin to form a chloroform soluble complex, which is measured on a colorimeter has been reported (31).

6.3 Infrared Analysis

An IR method of analysis for DSS has been proposed (32). This method involves precipitation of DSS as its barium salt with barium chloride, followed by analysis of its IR band at 9.5 μ .

6.4 Turbidimetric Analysis

A turbidimetric method of analysis based on addition of sodium chloride solution to a DSS solution with

measurement of subsequent turbidity at 320 nm has been reported (33).

6.5 Determination of Micellar Weight

Determination of micellar weights for DSS in anhydrous and hydrous hydrocarbon solutions has been made by means of light scattering (34).

6.6 Thin-layer Chromatography

DSS may be chromatographed on a Silica Gel G plate with ethyl acetate/ammonium hydroxide/ethanol (50/20/20) solvent system. Detection involves spraying with 50% sulfuric acid followed by heating the plate at 120°C for 1 hour (35).

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8. ACKNOWLEDGEMENTS

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FLUOROURACIL

Bruce C. Rudy and Bernard Z. Senkowski

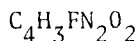
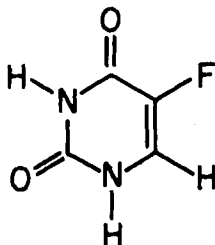
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1. Description

1.1 Name, Formula, Molecular Weight Fluorouracil is 5-fluorouracil.



Molecular Weight: 130.08

1.2 Appearance, Color, Odor

Fluorouracil is a white to practically white, practically odorless, crystalline powder.

2. Physical Properties

2.1 Infrared Spectrum (IR)

The IR spectrum of reference standard fluorouracil* is shown in Figure 1 (1). The spectrum of a mineral oil suspension of fluorouracil between cesium iodide discs was measured on a Perkin Elmer 621 Spectrophotometer. The assignments given in Table I to the bands in Figure 1 (1) are in good agreement with the assignments made by Brownlie (2).

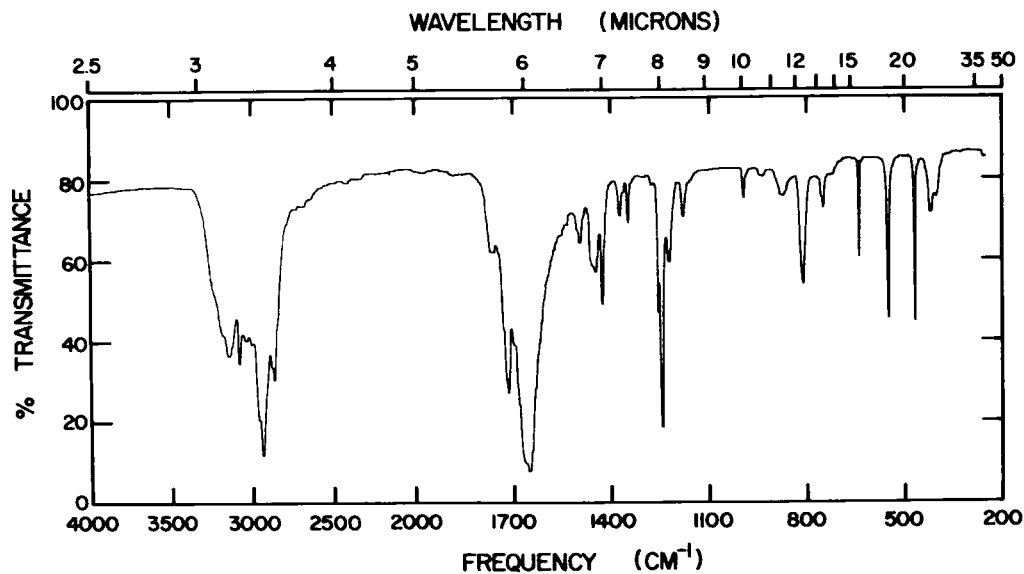
TABLE I

IR Spectral Assignments for Fluorouracil

<u>Characteristic of</u>	<u>Frequency (cm⁻¹)</u>
NH stretch	3124
C=O stretch	1716 and 1657
CH in plane deformation	1245
CH out of plane deformation	813

*The reference standard fluorouracil referred to in this work is No. 354029.

Figure 1
Infrared Spectrum of Fluorouracil



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2.2 Nuclear Magnetic Resonance Spectrum (NMR)

2.21 Proton Spectrum

The NMR spectrum shown in Figure 2 was obtained by dissolving 56.3 mg of reference standard fluorouracil in 0.5 ml of dimethylsulfoxide - d_6 containing tetramethylsilane as the internal reference (3). The spectral assignments are shown in Table II (3).

TABLE II

NMR Spectral Assignments for Fluorouracil

<u>Type Proton</u>	<u>No. of Each Proton</u>	<u>Chemical Shift (ppm)</u>	<u>Multiplicity</u>
C-H	1	7.80	d, ($J_{H-F}=6.5$ Hz)
N-H	2	11.40	S(b)

d= doublet; S(b) = broad singlet; J= splitting constant in Hz

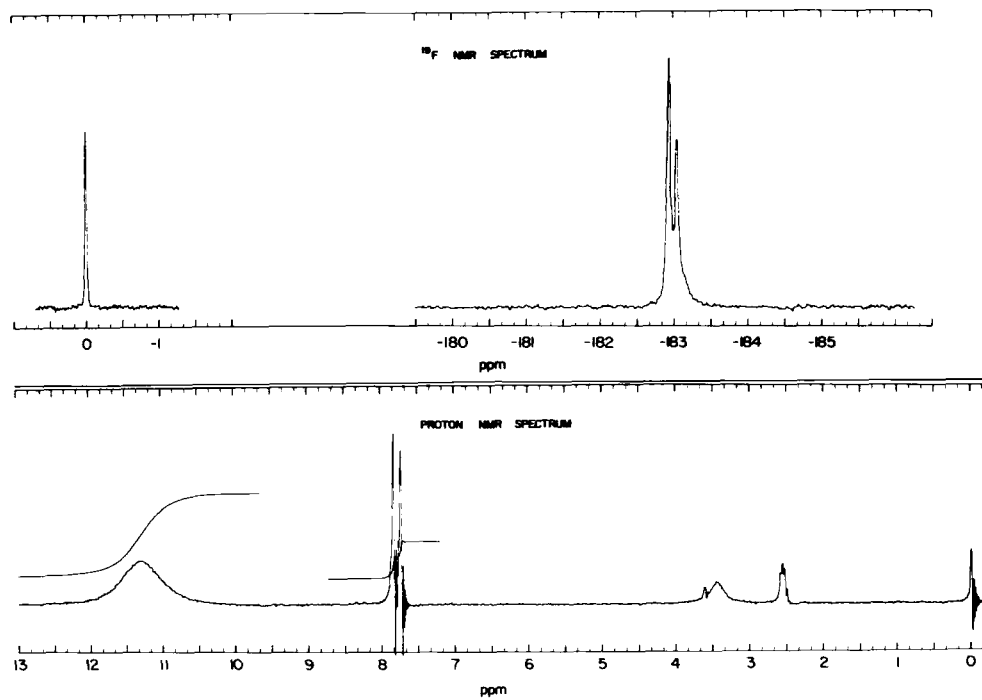
2.22 ^{19}F Spectrum

The ^{19}F spectrum shown in Figure 2 was obtained with a Jeolco C-60 HL instrument with ^{19}F module crystal modified to a frequency of 56.446 MHz. Seventy mg of reference standard fluorouracil were dissolved in 0.5 ml of dimethylacetamide containing CCl_3F as the internal reference (3). The spectrum consists of a doublet at -183 ppm which has a splitting constant (J_{F-H}) of 6.5 Hz. The choice of CCl_3F as the internal reference along with the assignment of -183 ppm is in accordance with Bovey (4).

2.3 Ultraviolet Spectrum (UV)

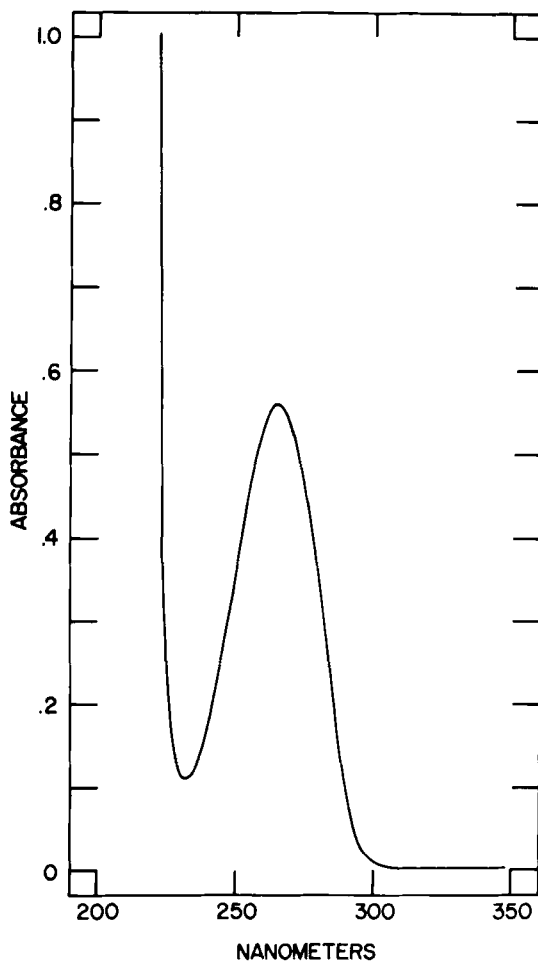
The UV spectrum of fluorouracil in acetate buffer (pH 4.7) in the region 350 to 220 nm exhibits one maximum at 266 nm ($\epsilon = 7.07 \times 10^4$) and one minimum at 232 nm. The spectrum presented in Figure 3 was obtained using a solution of 1.004 mg fluorouracil per 100 ml of pH 4.7 acetate buffer (5).

Figure 2



FLUOROURACIL

Figure 3
Ultraviolet Spectrum of Fluorouracil



2.4 Fluorescence Spectrum

Figure 4 shows the excitation and emission spectra for reference standard fluorouracil from 250 to 600 nm (6). The spectra, measured in a methanol solution of fluorouracil (1.0 mg/ml) using a Farrand MK-1 Spectrofluorometer, showed one excitation peak at 315 nm and one emission peak at 391 nm.

2.5 Mass Spectrum

The mass spectrum of fluorouracil shown in Figure 5 was obtained using a CEC-21-110 mass spectrometer with an ionizing energy of 70 eV (7).

The spectrum shows a strong molecular ion peak at m/e 130. The peak at m/e 97 is due to the loss of HNCO from the parent and the fragment at m/e 60 is formed by a hydrogen rearrangement giving half the ring with the fluorine substituent (C_2H_3FN) (7).

2.6 Optical Rotation

Fluorouracil exhibits no optical activity.

2.7 Melting Range

The melting range for fluorouracil depends on the rate of heating. When the USP XVIII Class I procedure (8) is used, the melting point lies between 280° and 284°C.

2.8 Differential Scanning Calorimetry (DSC)

The DSC curve shown in Figure 6 for reference standard fluorouracil was obtained using a Perkin Elmer DSC - 1B Calorimeter. With a temperature program of 10°C/min., a melting endotherm was observed starting at 288.2°C with a $\Delta H_f = 6.7$ Kcal/mole. The exotherm starting at 298.5°C corresponds to the decomposition of the fluorouracil (9).

2.9 Thermogravimetric Analysis (TGA)

The TGA performed on reference standard fluorouracil exhibited no loss of weight when heated from room temperature to 105°C at a heating rate of 10°C/min. (9).

Figure 4
Fluorescence Spectra of Fluorouracil

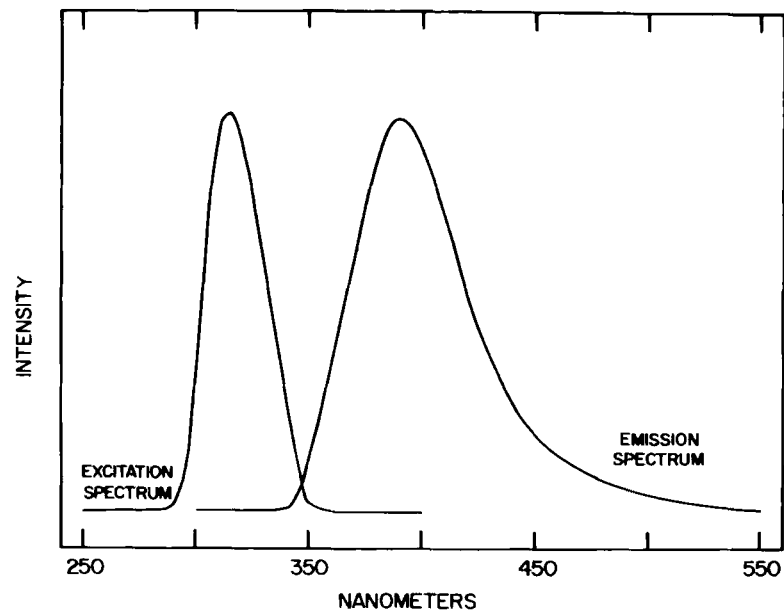
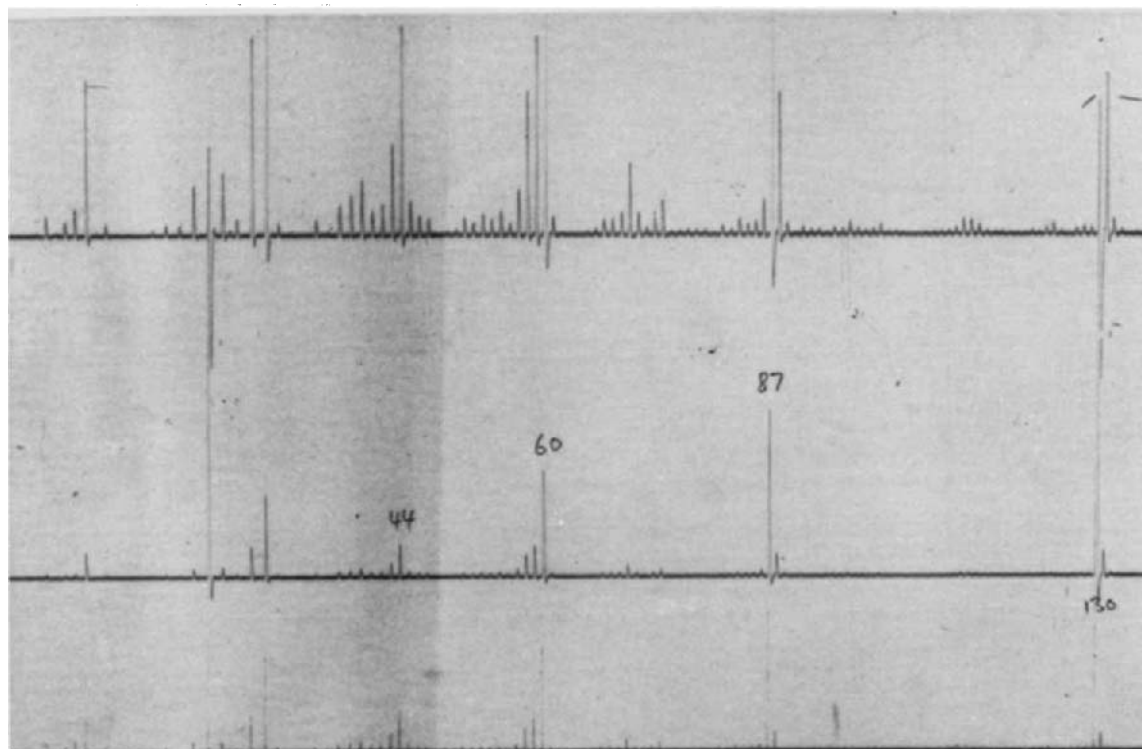


Figure 5

Mass Spectrum of Fluorouracil

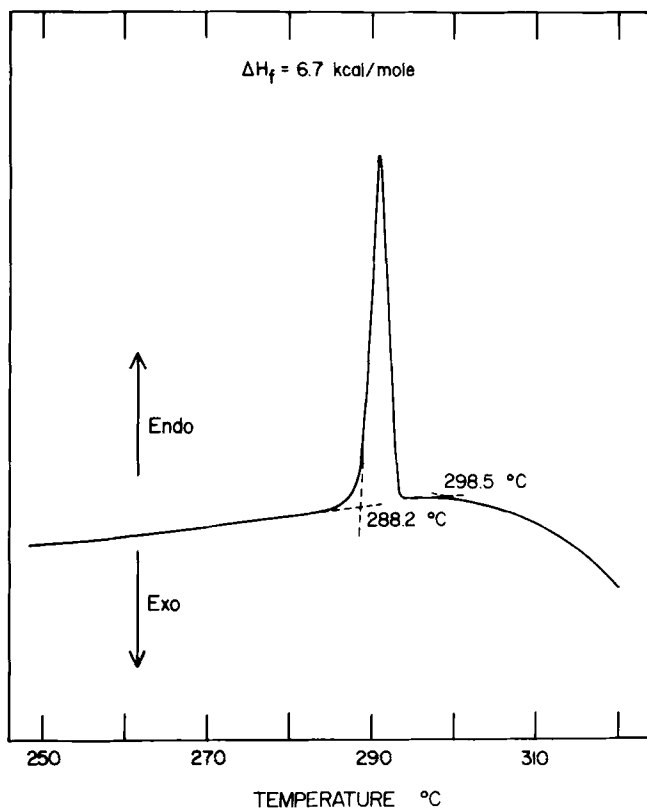


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FLUOROURACIL

Figure 6

D.S.C. Curve for Fluorouracil



2.10 Solubility

The solubility data obtained at 25°C for reference standard fluorouracil is listed in Table III (10).

TABLE III

Fluorouracil Solubility

<u>Solvent</u>	<u>Solubility (mg/ml)</u>
3A alcohol	3.44
benzene	<.1
chloroform	<.1
95% ethanol	5.54
ethyl ether	<0.1
isopropyl alcohol	2.15
methanol	9.37
petroleum ether (30°-60°)	<0.1
water	12.20

2.11 Crystal Properties

The x-ray powder diffraction pattern of reference standard fluorouracil is presented in Table IV (11). The instrumental conditions are given below:

Instrumental Conditions:

General Electric Model XRD-6 Spectrogoniometer

Generator:	50 KV 12.5 MA
Tube Target:	Copper
Radiation:	Cu K α = 1.542 Å
Optics:	0.1° Detector slit
	M.R. Soller slit
	3° Beam slit
	0.0007" Ni filter
	4° take off angle
Goniometer:	Scan at 0.2° 2 θ per minute
Detector:	Amplifier gain - 16 coarse,
	8.7 fine, Sealed proportional
	counter tube and DC voltage
	at plateau, Pulse height se-
	lection E _L -5 volts; E _H - out
	Rate meter T.C.4, 2000 C/S
	full scale

FLUOROURACIL

Recorder: Chart speed 1" per 5 minutes
 Samples: Prepared by grinding at room temperature

TABLE IV

X-ray Diffraction Pattern of Fluorouracil

<u>2θ</u>	<u>d$\overset{\circ}{A}$*</u>	<u>I/I$_0$**</u>
12.84	6.89	2
13.32	6.65	1
15.78	5.62	4
16.10	5.50	4
17.72	5.01	2
18.90	4.70	4
19.62	4.52	2
20.38	4.36	5
21.72	4.09	10
22.30	3.99	10
22.72	3.91	4
23.60	3.77	5
24.64	3.61	5
25.28	3.52	9
26.70	3.34	4
27.84	3.20	28
28.46	3.14	100
30.98	2.89	17
31.80	2.81	8
32.72	2.74	4
33.88	2.65	<1
34.84	2.58	2
36.20	2.48	2
37.01	2.43	2
37.54	2.40	<1
38.72	2.33	<1
39.14	2.30	<1
42.88	2.11	<1
44.08	2.05	<1
45.54	1.99	<1
46.52	1.95	<1
47.26	1.92	1
50.06	1.82	1
50.78	1.80	1

53.56	1.71	<1
54.28	1.69	2

$$*d = (\text{interplanar distance}) \frac{n\lambda}{2 \sin \theta}$$

**I/I₀ = relative intensity (based on highest intensity of 1.00)

2.12 Dissociation Constant

The pK_a's for fluorouracil have been determined spectrophotometrically to be 8.0 + 0.1 and 13.0 + 0.1 (12). When the pK_a's for uracil were determined in a similar manner, they were found to be 9.4 + 0.1 and >13.5. These latter values are in good agreement with the pK_a's reported by Shugar and Fox for uracil (13).

3. Synthesis

Fluorouracil may be prepared by the reaction scheme shown in Figure 7. Ethyl fluoroacetate is condensed in a Claisen condensation with methyl formate. The ethyl sodium fluormalonaldehyde formed is condensed with 2-ethyl-2-thiopseudourea hydrobromide to give 2-ethylmercapto-5-fluor-4(3H)-pyrimidinone which yields fluorouracil on hydrolysis (14).

4. Stability Degradation

Fluorouracil is stable in solutions which are not strongly basic (pH less than 9). When subjected to strongly basic conditions, the fluorouracil is hydrolyzed to urea, fluoride, and an aldehyde. This hydrolysis is enhanced by increased pH and temperature. Some of the urea formed on hydrolysis reacts further giving ammonia and CO₂ (15).

5. Metabolic Products

The metabolic pathway of fluorouracil is presented schematically in Figure 8. In man, the major biochemical effect of fluorouracil is the inhibition of DNA synthesis, since concentrations which inhibit DNA synthesis may still permit RNA synthesis. Fluorouracil is converted to fluorouridine and then to the mono-, di-, and triphosphates of fluorouridine. This is then incorporated into the fraudulent RNA. Fluorouridine monophosphate is also reduced to fluoro-2'-deoxyuridine monophosphate. There is no further metabolism to the di- and triphosphate nucleotides

Figure 7

SYNTHESIS OF FLUOROURACIL

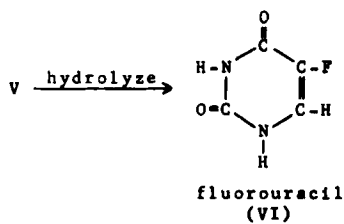
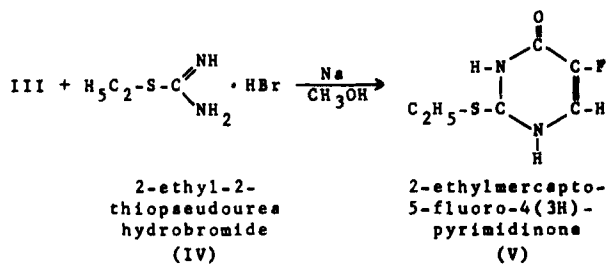
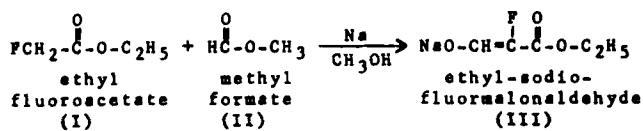
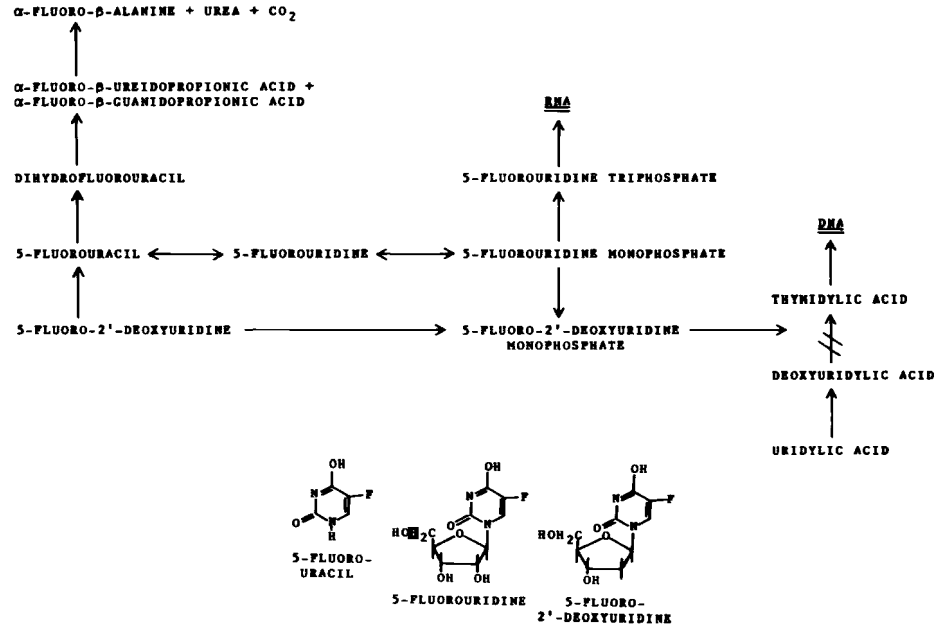


Figure 8

METABOLIC PATHWAYS OF FLUOROURACIL (16)



of fluoro-2'-deoxyuridine. Therefore the formation of the fluoro-2'-deoxyuridine monophosphate is considered to be the basis for the antineoplastic action of fluorouracil since it inhibits DNA synthesis by blocking the enzyme thymidylate synthetase. It is this enzyme that catalyzes the methylation of deoxyuridylic acid to thymidylic acid (16).

Fluorouracil is catabolized in an analogous manner to uracil, forming the following degradative products: dihydrofluorouracil, α -fluoro- β -ureidopropionic acid, α -fluoro- β -guanidopropionic acid, α -fluoro- β -alanine, urea, and CO_2 (16).

6. Methods of Analysis

6.1 Elemental Analysis

The results from an elemental analysis of reference standard fluorouracil is presented in Table V (17).

TABLE V

Elemental Analysis of Fluorouracil

<u>Element</u>	<u>% Theory</u>	<u>% Found</u>
C	36.93	36.94
H	2.32	2.50
F	14.61	14.57

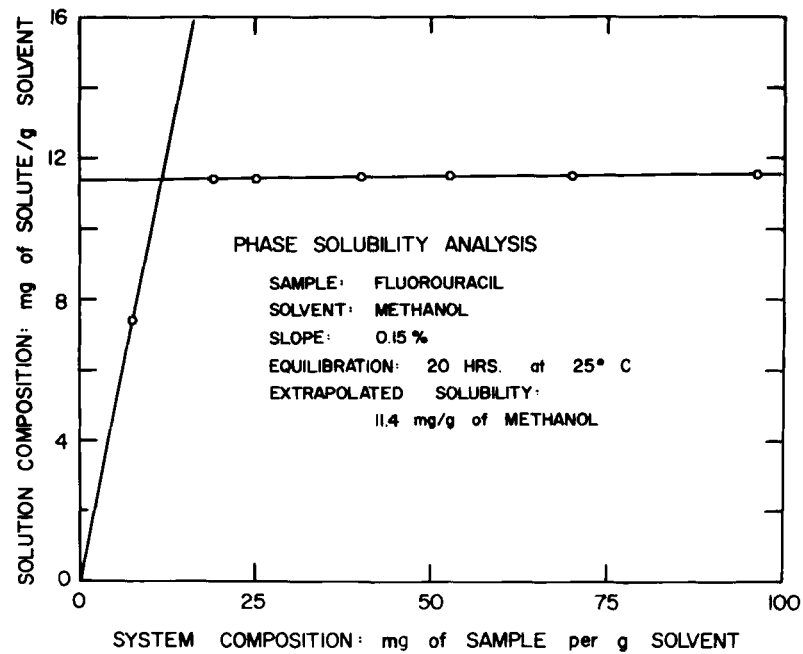
6.2 Phase Solubility Analysis

Phase solubility analysis has been carried out for fluorouracil using methanol as the solvent. An example is presented in Figure 9 for a typical lot of fluorouracil along with the conditions under which the analysis was carried out (10).

6.3 Thin Layer Chromatographic Analysis (TLC)

The TLC of fluorouracil and other fluoro-pyrimidines has been studied at length by Hawrylyshyn, Senkowski, and Wollish (18). The R_f values presented in Table VI were obtained in various developing solvents when 10 μgm of fluorouracil was spotted on a silica gel GF

Figure 9



FLUOROURACIL

plate. After development for at least 10 cm the plates were air dried and viewed under short wave ultraviolet radiation.

TABLE VI

R_f Values for Fluorouracil
in Various Developing Solvents (18)

<u>Solvent System</u>	<u>R_f Value</u>
ethyl acetate:acetone:water (70:40:10)	0.7
ethyl acetate:methanol:conc. ammonium hydroxide (75:25:1)	0.4
acetone	0.8
ether	0.12
ethyl acetate	0.4
methanol	0.8
2-propanol	0.7
ethyl acetate:methanol (80:20)	0.8
ethyl acetate:methanol (75:25)	0.7
ethyl acetate:water (100:1)	0.2
ethyl acetate:water (100:3)	0.4
ethyl acetate:methanol:glacial acetic acid (75:25:1)	0.9
chloroform:glacial acetic acid (65:35)	0.45

6.4 Gas Liquid Chromatographic Analysis (GLC)

GLC has been carried out for fluorouracil using the instrumental conditions listed below. A single peak is observed for reference standard fluorouracil after about two minutes (19).

Instrumental Conditions:

Column:	6 ft. copper tubing
Column Packing:	5% SE-30 on Chromosorb G
Column Temperature:	250°C
Carrier Gas:	Nitrogen
Flow Rate:	40 cc/minute
Detector:	Hydrogen Flame Ionization

6.5 Direct Spectrophotometric Analysis

Direct spectrophotometric analysis is carried out on fluorouracil injectable solutions. A volume of the fluorouracil injection is pipetted into a volumetric flask and diluted with pH 4.7 acetate buffer to obtain a final concentration of about 10 µgm/ml. The absorbance of this solution is measured at 266 nm along with the absorbance of a similar concentration of reference standard fluorouracil. From this data the concentration of fluorouracil in the injectable solution is calculated (20).

6.6 Fluorine Analysis6.61 Organically Bound Fluorine Analysis

There are several methods available to determine the amount of carbon-bonded fluorine. One of the earlier methods employed the Schöniger Combustion technique followed by thorium nitrate or cerous chloride titration using sodium alizarin sulfonate or murexide as the indicator (21).

With the advent of good specific ion electrodes, methods were developed to liberate the bound fluorine and directly measure the fluoride concentration. The reagent sodium-biphenyl followed by oxidation with hydrogen peroxide is used to liberate the organically bound fluorine in fluorouracil. A fluoride specific ion electrode is used, in conjunction with a high-ionic-strength-buffer solution, for direct measurement of the liberated fluoride (22).

The last method to be presented for the analysis of the carbon-bonded fluorine in fluorouracil is ¹⁹F Nuclear Magnetic Resonance spectrometry (3). A fluorouracil reference standard and an internal standard,

reagent grade orthofluorobenzoic acid, is dissolved in N,N-dimethylacetamide and the ^{19}F spectrum obtained. From this data an internal standard fluorine conversion factor is obtained as follows: $A_b \times C_f \times 14.62 \times P$

$$\frac{A_b \times C_f \times 14.62 \times P}{A_f \times C_b} = CF \text{ where:}$$

A_b = height of orthofluorobenzoic acid signal

A_f = height of reference standard fluorouracil signal

C_f = mg/ml of reference standard fluorouracil

C_b = mg/ml of orthofluorobenzoic acid

14.62 = theoretical fluorine content in fluorouracil

$P = \frac{\text{assay of fluorouracil reference standard}}{100} \approx 1$

CF = internal standard fluorine conversion factor

Now a ^{19}F spectrum of each fluorouracil sample is obtained and the % fluorine calculated using the following formula.

$$\frac{A_s \times C_b \times CF}{A_b \times C_b} = \%$$

where: A_s = height of fluorouracil sample signal

C_s = mg/ml of fluorouracil sample

6.62 Free Fluoride Analysis

The determination of any free fluoride present in fluorouracil bulk samples or in fluorouracil ampuls are carried out by direct measurement using a fluoride specific ion electrode. The measurements are made in a tris(hydroxymethyl)amino-methane buffer solution (pH = 7.5). The electrode response was found to be linear throughout the working range of 0.08 to 0.25 mg $\text{F}^{(-)}/100 \text{ ml}$ solution (23).

6.7 Volumetric Analysis

Fluorouracil may be assayed by dissolving an accurately weighed sample in dimethylformamide and titrating it with 0.1N tetra-n-butyl-ammonium hydroxide in benzene to a blue end point using thymol blue as the indicator.

Each milliliter of 0.1N tetra-n-butylammonium hydroxide is equivalent to 13.01 mg of fluorouracil (24).

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FLUPHENAZINE ENANTHATE

Klaus Florey

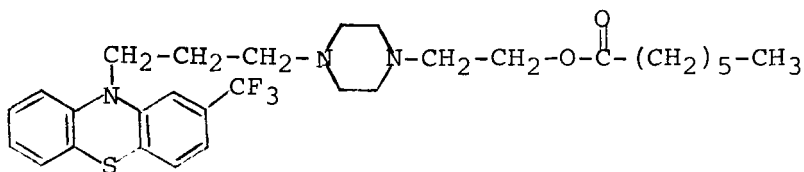
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IN BODY FLUIDS AND TISSUE
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1. Description

1.1 Name, Formula Molecular Weight

Fluphenazine enanthate is 4-(2,6-bis(trifluoromethyl)-1,2,3,4-tetrahydrophthalazin-1-yl) piperazine ethanol heptanoate (ester); also fluphenazine heptanoate, SQ 16,144; dihydrochloride salt: SQ 10,479



$C_{29}H_{38}F_3N_3O_2S$

Mol. Wt. 549.70

1.2 Appearance, Odor, Color

Fluphenazine enanthate is a pale yellow to yellow orange, clear to slightly turbid, viscous liquid with a characteristic odor.

2. Physical Properties

2.1 Infra Red Spectra

The infra red spectra of a semi-solid smear and of a chloroform solution are presented in figures 1 and 2.

2.2 Nuclear Magnetic Resonance Spectra

The NMR Spectrum is presented in figure 3. The following assignments can be made for chemical shifts of the spectrum of the free base².

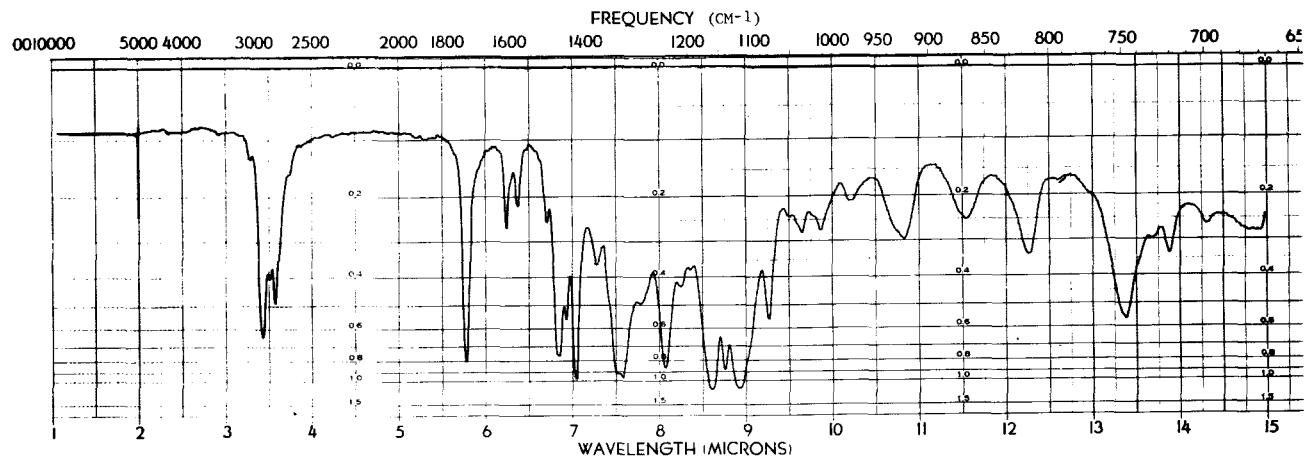


Figure 1 Infrared Spectra of Fluphenazine enanthate, Squibb House Standard batch #10 semisolid smear. Instrument: Perkin-Elmer 621. Curve #29377

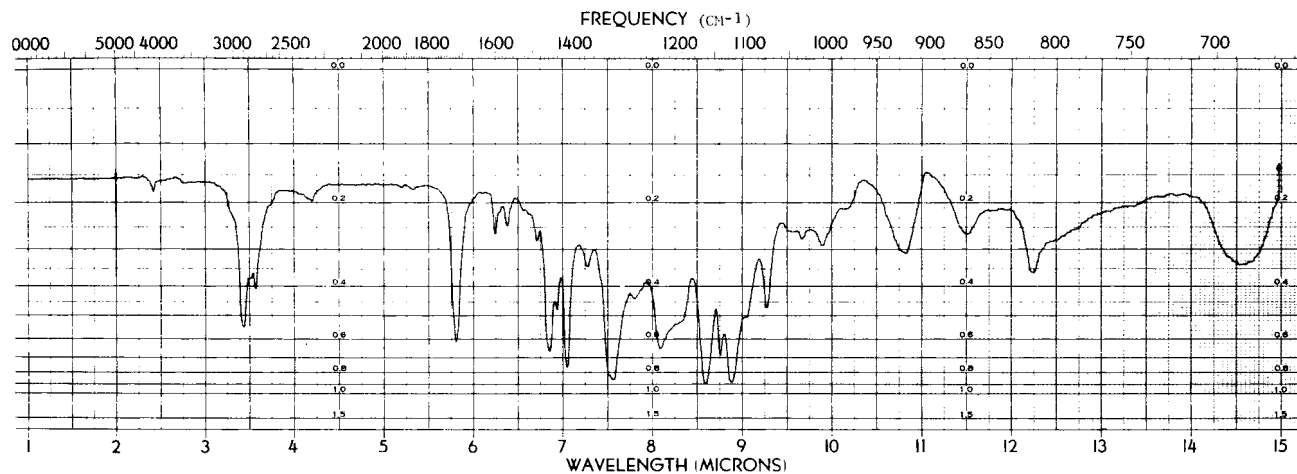


Figure 2 Infra Red Spectrum of Fluphenazine enanthate
Squibb House Standard batch #10 in chloroform solution.
Instrument: Perkin-Elmer 621. Curve # 29377A

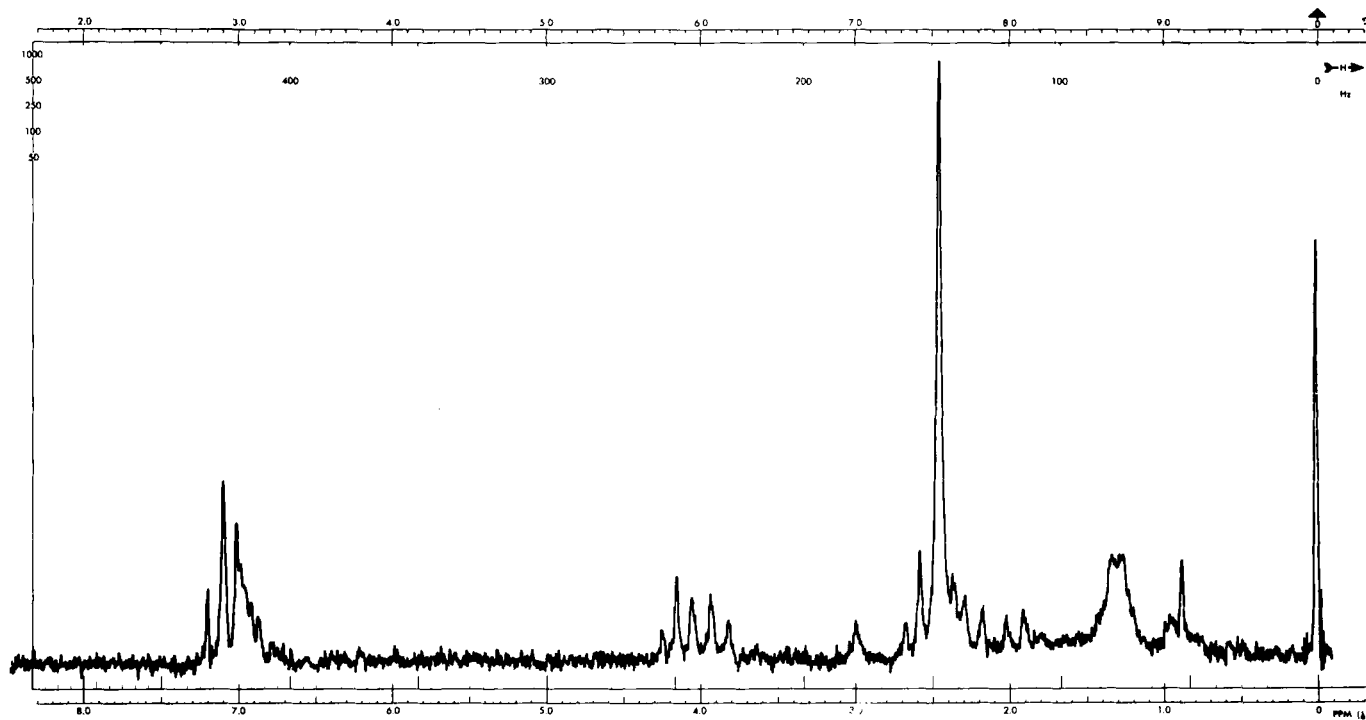


Figure 3: NMR Spectrum of fluphenazine enanthate, Squibb batch #10 in CDCl_3 . Spectrum # 10450. Instrument: Varian A-60.

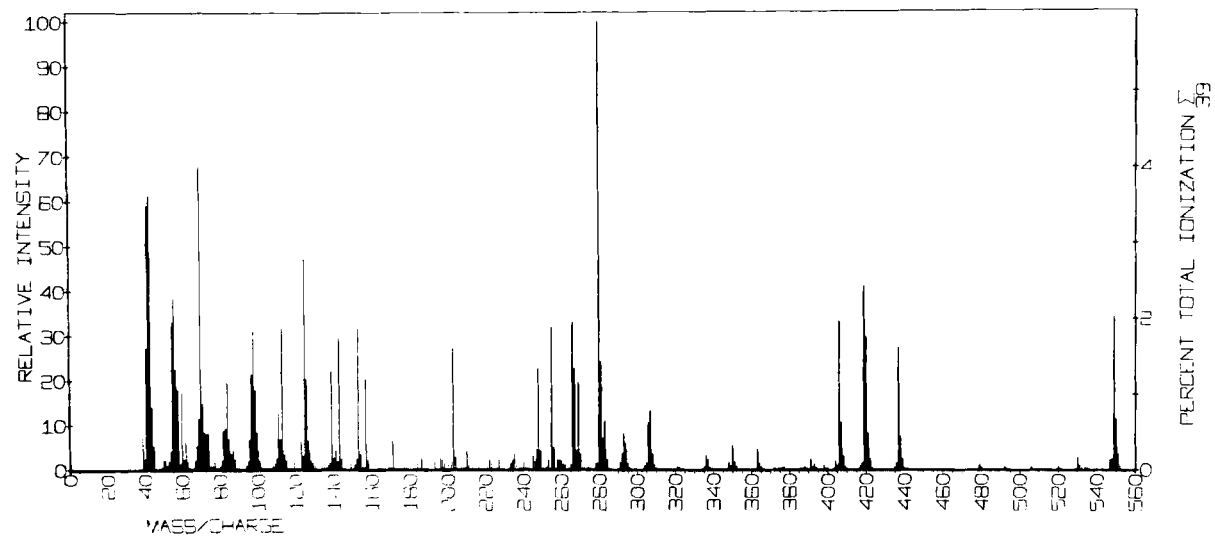
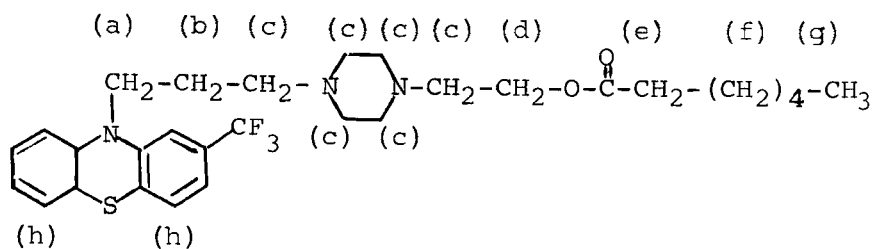


Figure 4: Low Resolution Mass Spectrum of Fluphenazine enanthate. Squibb House Standard batch 10. Instrument:MS-9.



Proton	Chemical Shifts ()	
a	6.05	triplet
b	8.07	multiplet
c	7.55	absorption envelope
d	5.84	triplet
e	8.10	triplet
f	8.70	multiplets
g	9.10	multiplet
h	3.0	aromatic

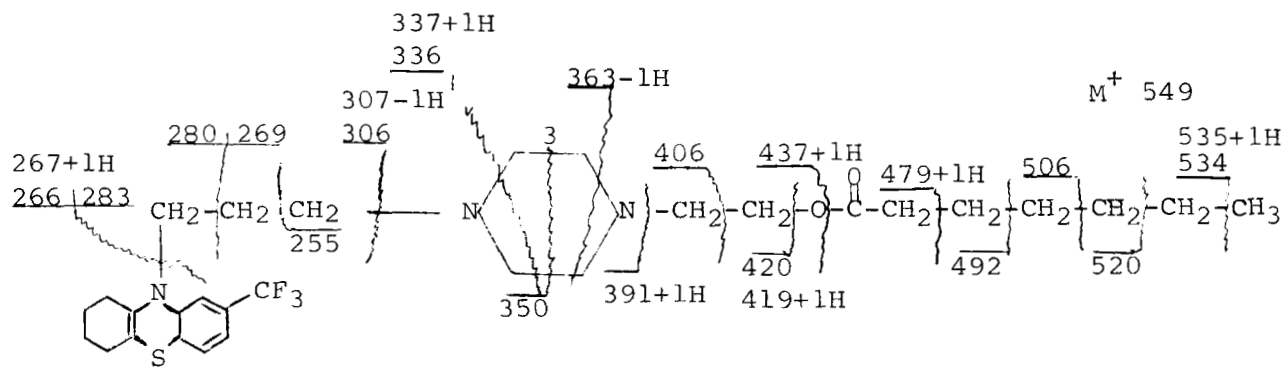
2.3 Ultraviolet Spectrum

Squibb House Standard batch #7, curve #22272 in ethanol³

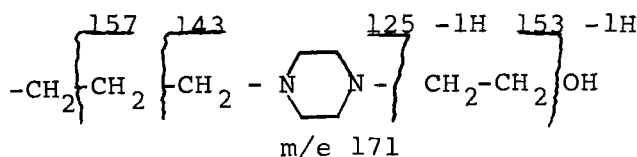
Wavelength (nm)	absorption ($E_{1\text{cm}}^{1\%}$)
240 (shoulder)	221
261	625
312	68

2.4 Mass Spectrum

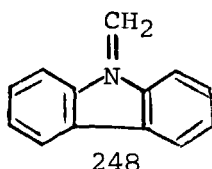
The low resolution mass spectrum is presented in figure 4. The spectrum indicates the following fragmentation pattern, consistent with that obtained for fluphenazine:



The side chain fragment and its further fragmentation products were also found:



Another prominent fragment is the following:



2.5 pKa, pH.

The pKa, value was determined by titration; the pKa₂ value was estimated from the pH of the first equivalent point because fluphenazine enanthate precipitated at pH values slightly above this point⁸.

	pKa,	pKa ₂
Fluphenazine enanthate	3.50;3.29	8.2;7.7
Fluphenazine	3.90;3.60	8.1;7.8

As expected the values are very similar to those of fluphenazine (see Analytical Profile), since the addition of an ester two carbons removed from the tertiary amine nitrogen should have a minimal effect⁸.

2.6 Boiling Point

Fluphenazine enanthate base is a viscous liquid and attempts to distill it and to determine a boiling point by conventional

methods have been unsuccessful⁶.

2.7 Differential Thermal Analysis

Fluphenazine enanthate samples subjected to DTA at atmospheric pressure under N₂ and in vacuo (25mm) showed neither exothermic or endothermic peaks up to a temperature of 350°C⁷.

2.8 Solubility

Solvent	Solubility gm/ml solvent at 25°C ⁸
Water	insoluble
Ethanol	<1 (v.s.)
Chloroform	<1 (v.s.)
Ether	2

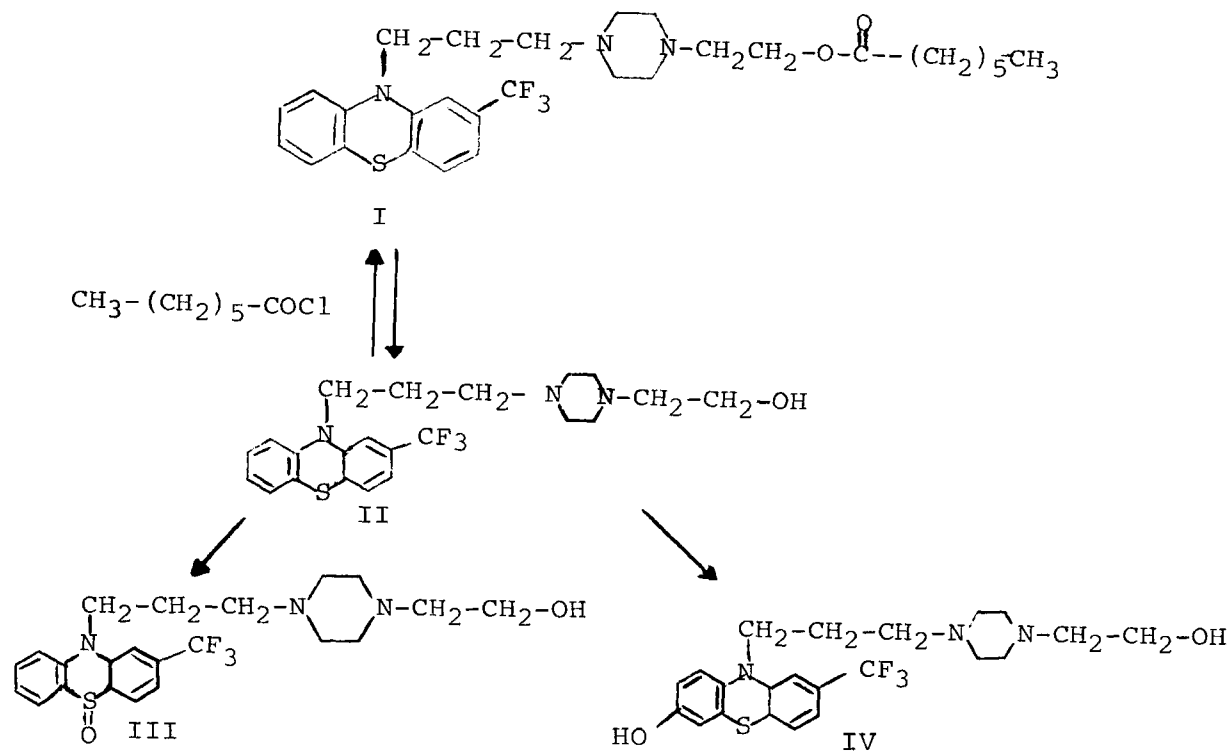
2.9 Refractive Index

The Refractive Index of Squibb House Standard batch #7 is determined as:
1.5495 (23°C)³

3. Synthesis

Fluphenazine enanthate (I, Figure 5) has been prepared by reacting fluphenazine (II) with heptanoyl chloride.^{9,10}

Figure 5. Chemistry of Fluphenazine Enanthate.



4. Stability-Degradation.

Fluphenazine enanthate, like its parent compound fluphenazine hydrochloride is light sensitive. Exposure of a 10% solution of fluphenazine enanthate in iso-butyl methyl ketone for one month resulted in 5% decomposition.¹¹ The mechanism of light catalyzed decomposition is probably similar to that of fluphenazine hydrochloride (see Analytical Profile). Fluphenazine enanthate hydrolyzes to fluphenazine in alkaline medium.

5. Drug Metabolic Product

The drug metabolism and tissue distribution of fluphenazine enanthate, labeled with C¹⁴ in the ethanol portion of the side chain was studied. In addition to unchanged fluphenazine enanthate two metabolites fluphenazine (II) and fluphenazine sulfoxide (III) were identified in the excreta and all the tissues studied. However in the brain only fluphenazine was found. The hydrolysis in the brain of fluphenazine enanthate to fluphenazine was demonstrated.¹²

In a later study the β -glucuronide of 7-hydroxyfluphenazine (IV) was isolated from dog bile after administration of ¹⁴C labeled fluphenazine enanthate.¹³

6. Method of Analysis

6.1 Elemental Analysis

	Calc.	Found ⁹
C	63.37	63.09
H	6.98	7.10
N	7.64	7.99

6.2 Nonaqueous Titration

Fluphenazine enanthate can be titrated with 0.1 N HClO₄ in glacial acetic acid. Indicator: crystal violet.¹⁴ Neutralization equivalent: calc. 285; Found: 271 (Squibb House Standard).

6.3 Spectrophotometric Analysis

The U.V. absorbance at 261 nm (see Section 2.3) can be used for quantitative analysis (200 section 6.71).

6.4 Spectrofluorometric Analysis

Although no data are available, spectrofluorometric analysis of fluphenazine enanthate should be possible, similar to that of the parent compound (see Analytical Profile on Fluphenazine Hydrochloride).

6.5 Colorimetric Analysis

The following color tests were applied¹¹:

Reagent:	Color:
50% Sulfuric acid	red
Nitroprusside	faint grey
Gibbs	grey
Hydroxamic acid	yellow-orange
Bromo-cresol green	blue
Phenol red	red
Phosphomolybdic acid	green grey- green yellow

6.6 Chromatographic Analysis

6.61 Paper chromatographic Analysis

The following systems have been reported.

Systems:	Fluphenazine enantate	Fluphenazine sulfoxide	Fluphena- zine
System 1 ¹²	0.96	0.55	0.10
System 2 ¹²	0.00	0.35	0.62
System 3 ¹⁰	0.13	0.68	--
System 1 ¹² :	Whatman paper #1 benzene - acetic acid - water (2:2:1)		
System 2 ¹² :	Whatman paper #3 MM 1N sodium formate (ascending)		
System 3 ¹⁶ :	Paper	Whatman#1 (ethanol washed)	
	Stationary phase:	Castor oil (2% in ether)	
	Developing solvent:	Methanol-water (85-15)	
	Development time:	16 hours	

This last system can be used for quantitation by locating the spots under U.V. light, elution with 95% ethanol and measuring U.V. absorbance at 261 nm against a standard,¹⁶ following the general procedure.¹⁷

6.62 Thin layer chromatography:

See Table I

6.63 Gas-liquid chromatography

Fluphenazine enanthate can be chromatographed isothermally at 250°C on 8/100 mesh glass beads coated with 0.5% of SE-30. With a retention time of 6-7 minutes the minimum detectable quantity is 0.5 μ gm.

Reproducible quantitation was not achieved - non - linear erratic response indicated absorption of the enanthate with possible attendant degradation.¹⁸

7. Identification and Determination in Body Fluids and Tissues.

Determination by spectrofluorometry in rat brain and plasma.¹⁵ Thin layer chromatography (see Section 5)^{12,13}.

Table I

Thin Layer Chromatography

The following systems have been reported.

		Rf values			
System		Fluphenazine enantate	Fluphenazine dihydrochloride	Fluphenazine sulfoxide	7-Hydroxy- fluphenazine
261	Benzene-ammonia- dioxane (60:5:35) ^{12,13}	0.84	0.26	0.06	0.05
	Chloroform-95% ethanol-ammonia (10:85:2.5) ¹³	0.87	0.75	--	0.66
	Chloroform-abs. ethanol-ammonia (80:10:1) ¹⁹	0.82	0.47	--	0.15
	Acetone-cyclohexane- ammonia (50:40:1) ¹¹	0.75	0.40	--	--

Silica gel GF thin layer plates were used. Detection and spray reagents.¹¹
U.V. light 50% sulfuric acid.

FLUPHENAZINE ENANTHATE

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FLUPHENAZINE HYDROCHLORIDE

Klaus Florey

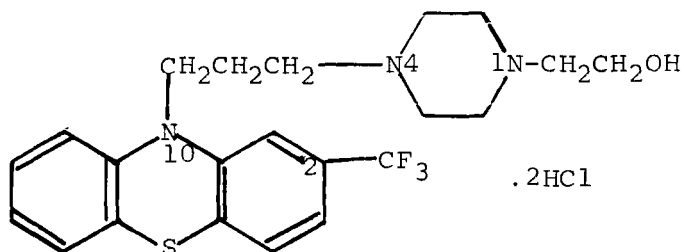
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1. DESCRIPTION

1.1 Name Formula, Molecular Weight

Fluphenazine Hydrochloride is 4-{3-[2-(trifluoromethyl)-phenothiazine-10-yl] propyl} piperazine ethanol dihydrochloride; also 4-(3-[10-2(-trifluoromethyl)-phenothiazinyl] propyl)-1-piperazine-ethanol dihydrochloride; 1-(2-hydroxy ethyl)-4-[2-trifluoromethyl-10-phenothiazinyl] propyl] piperazine dihydrochloride; 10-(3'-[4'-(β-hydroxy ethyl)-1'-piperazinyl] propyl-2-trifluoromethyl-phenothiazine dihydrochloride; S94; SQ 4918.

C₂₂H₂₆F₃N₃OS.2HCl

Mol. Wt. 510.45

1.2 Appearance, Color, Odor

White to off-white, odorless crystalline powder.

2. PHYSICAL PROPERTIES

2.1 Infrared Spectra

The infrared spectra of Squibb House Standard #48101-001, in mineral oil mull and KBr from MeOH, are presented in figures 1 and 2.¹ The infrared spectrum of the free base, and a discussion of spectra-structure correlation of phenothiazines have been presented.^{2,3} The spectra published by Sammul, et al.⁴ are in essential agreement with those presented here.

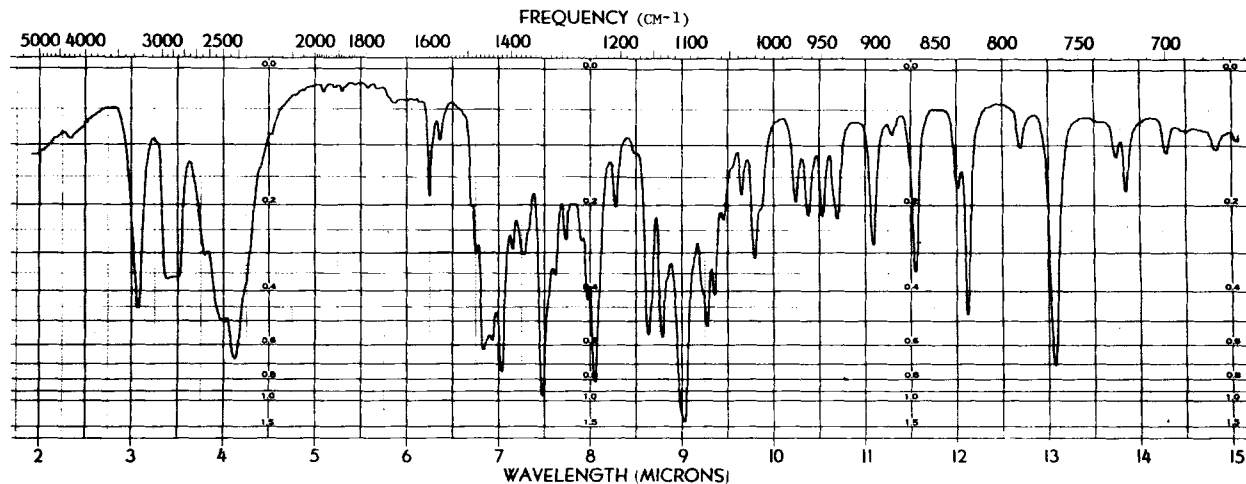


Fig. 1 Infrared Spectrum of fluphenazine hydrochloride in mineral oil mull. Instrument: Perkin-Elmer 621

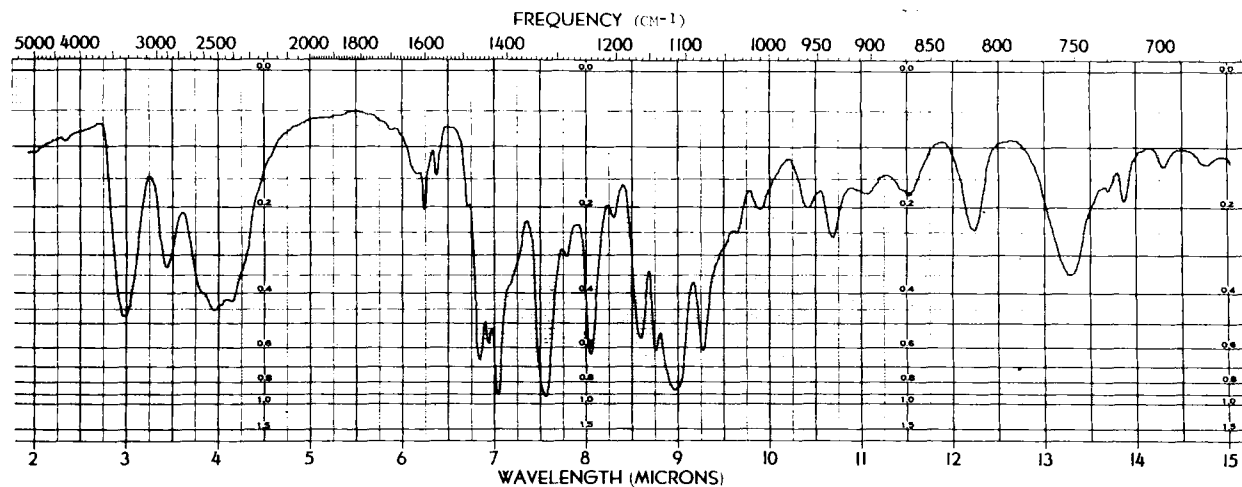
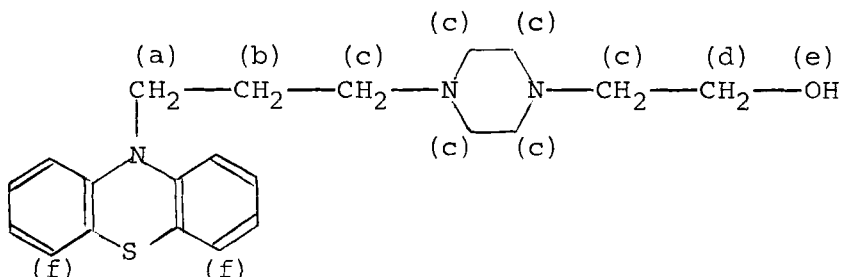


Fig. 2 Infrared Spectrum of fluphenazine hydrochloride in KBr from methanol. Instrument: Perkin-Elmer 621

2.2 Nuclear Magnetic Resonance Spectra

The NMR spectra of fluphenazine base and hydrochloride are presented in figures 3 and 4.⁵ The spectrum of the free base is essentially identical with that published previously.³ The following assignments can be made for chemical shift of the spectrum of the free base.⁶



Proton Position	Chemical Shift ()	
a	6.05	triplet
b	8.09	multiplet
c	7.57	absorption envelope
d	6.41	triplet
e	6.93	singlet
f	2.9-3.0	aromatic

2.3 Ultraviolet Spectra

The following U.V. data have been recorded:

1. in methanol¹ (Instrument Cary 15)

$$\lambda_{\max} 261 \text{ m}\mu \quad E_{1\text{cm}}^{1\%} 646$$

$$\lambda_{\max} 310 \text{ m}\mu \quad E_{1\text{cm}}^{1\%} 69$$

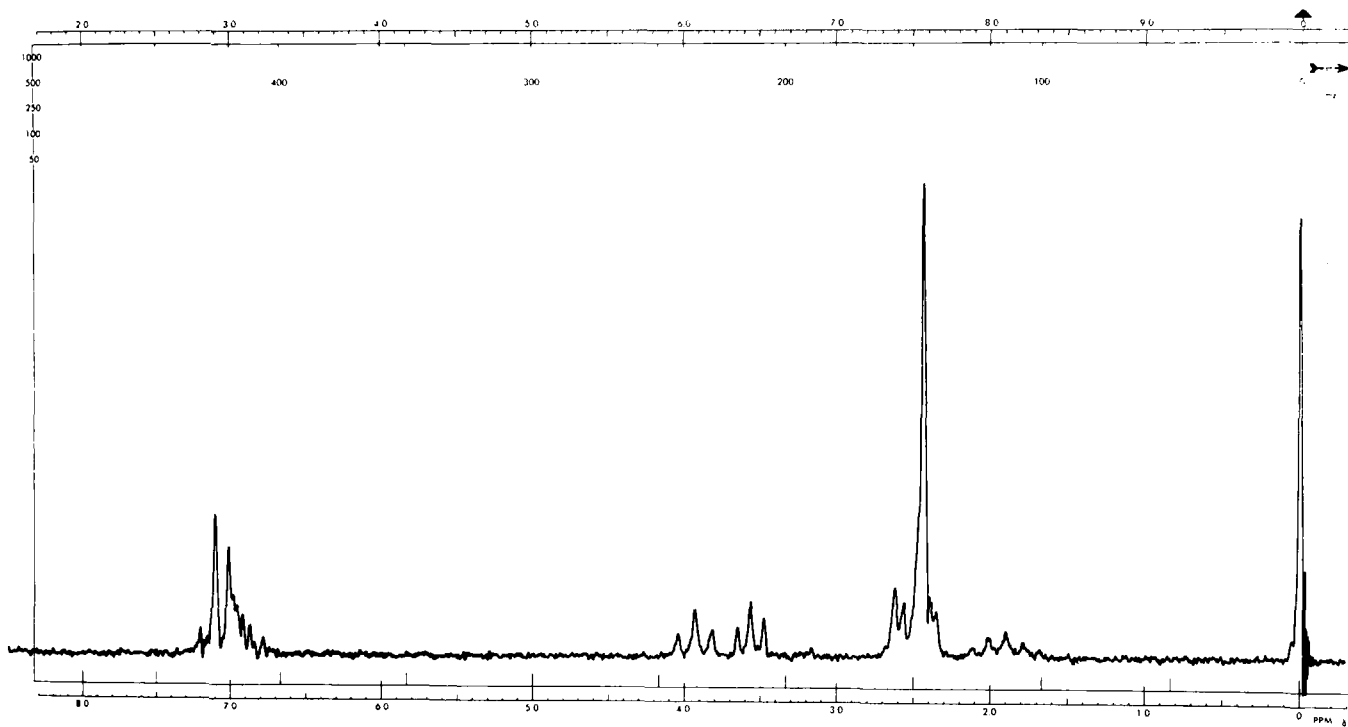


Fig. 3 NMR Spectrum of fluphenazine base in deuteriochloroform.
Instrument: Varian A-60

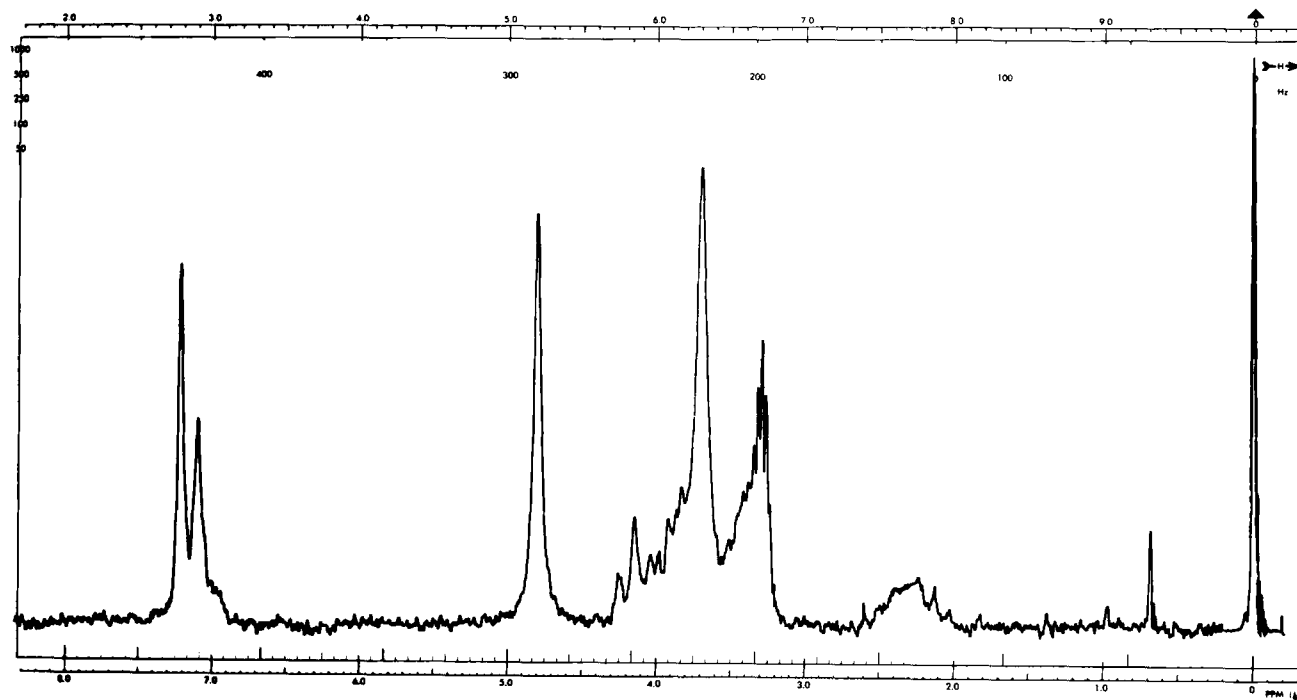


Fig. 4 NMR Spectrum of fluphenazine hydrochloride in deuteriochloroform.
Instrument: Varian A-60

2.3 Ultraviolet Spectra Cont'd.

2. in 95% ethanol³

λ_{\max} 264 Log ϵ 4.59

λ_{\max} 316 Log ϵ 3.69

λ_{\min} 239 Log ϵ 4.28

λ_{\min} 300 Log ϵ 3.51

3. in 50% alcohol (Instrument Beckman

DU)⁷

$E_{1\text{cm}}^{1\text{ppm}}$

λ_{\max} 259 0.052

λ_{\max} 305 0.006

λ_{\min} 224 0.013

λ_{\min} 280 0.002

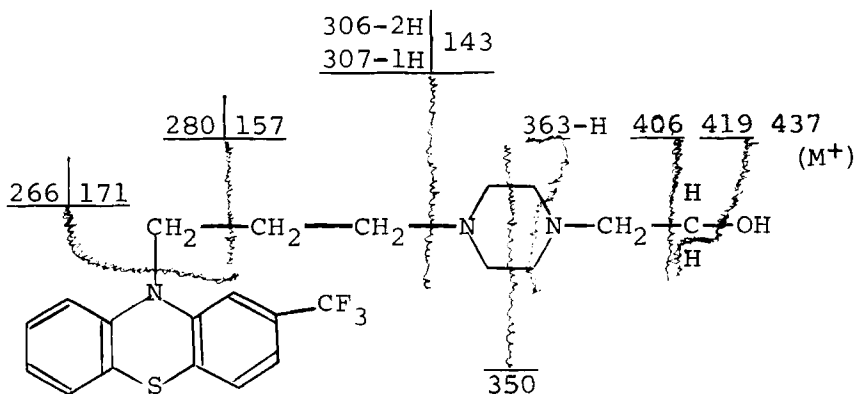
4. in methanol⁸

λ_{\max} 259 Log ϵ 4.53

2.4 Mass Spectra

The low-resolution mass spectrum of fluphenazine base is represented in figure 5.⁹

The high-resolution spectrum shows the following fragmentation pattern of side chain and nucleus⁹, which is in agreement with the results of an independent investigation.⁶⁹



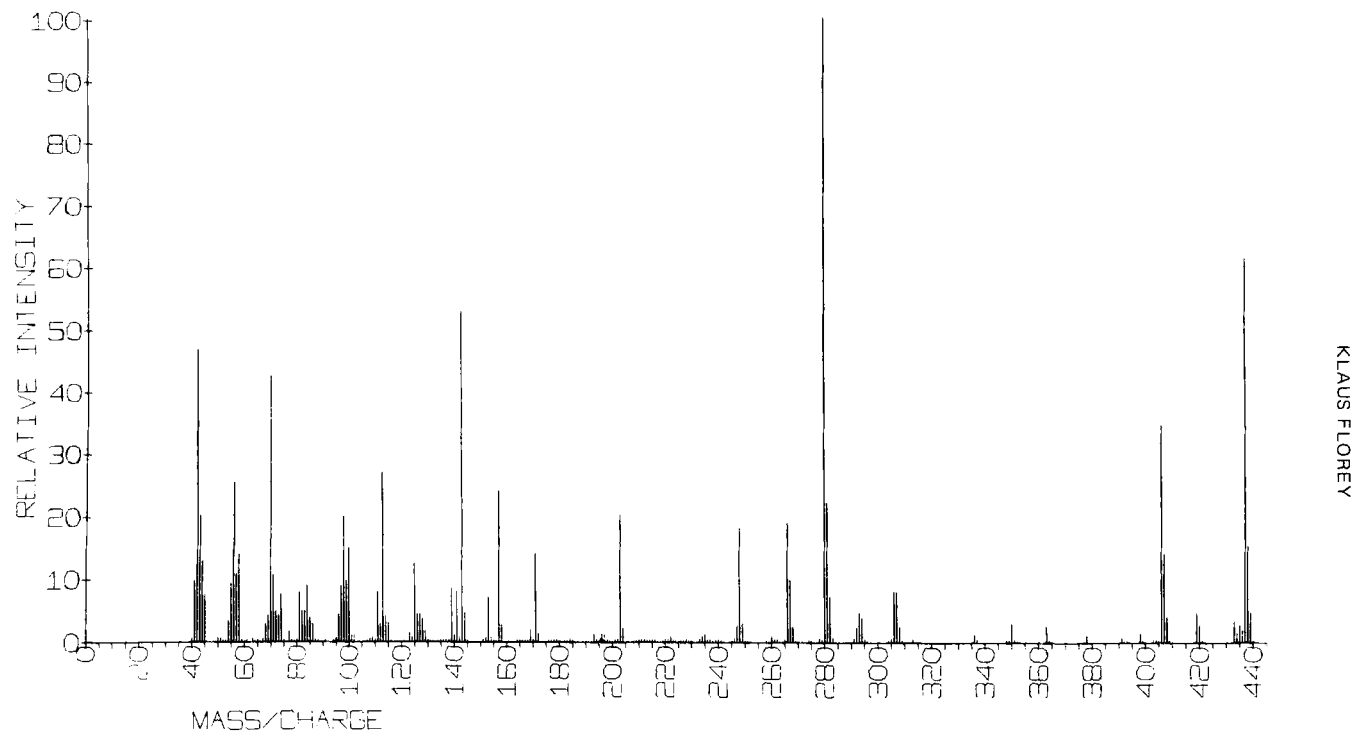
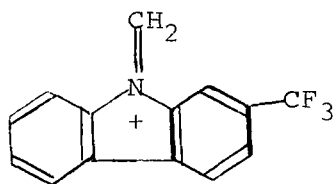


Fig. 5 Low-resolution Mass Spectrum of fluphenazine. Instrument: MS-9

Another prominent fragment is the following:



248

The significant mass numbers shown above can be tabulated as follows:

									Mass Units Diff.	
	C	H	N	O	S	F	Unsat	O/E*	Found	- Calculated
437	22	26	3	1	1	3	10	0		0.3
419	22	24	3	-	1	3	10	0		-0.7
406	21	23	3	-	1	3	10.5	E		-1.6
363	19	18	2	-	1	3	10.5	E		-0.3
307	16	12	1	-	1	3	10	0		-1.1
306	16	11	1	-	1	3	10.5	E		-0.3
280	14	9	1	-	1	3	9.5	E		-0.1
266	13	7	1	-	1	3	9.5	E		0.5
248	14	9	1	-	-	3	9.5	E		-0.1
171	9	19	2	1	-	-	1.5	E		-0.1
157	8	17	2	1	-	-	1.5	E		-0.7
143	7	15	2	1	-	-	1.5	E		-0.1

*0 = odd electron ion; E = even electron ion

2.5 pKa; pH

The apparent pKa of fluphenazine dihydrochloride in aqueous alcoholic solution has been determined as 8.05 for the second basic group. For the first basic group, a value of 3.90 was obtained.¹⁰ Independently, values of 7.8 (estimated) and 3.60, respectively, were obtained.⁷¹

The pH of an aqueous solution of the dihydrochloride is 2.4; that of monohydrochloride is

5.5.11

2.6 Melting Range

The following melting points of the hydrochloride have been reported:

238.5-239.5° (Recryst. from abs. ethanol USP Method)¹¹
 231-233° (Recryst. from abs. ethanol)¹²
 224-226°^{13,14}
 224.5-226° (Recryst. from methanol-ether)¹⁵
 235-237°^{16,17}
 231-232°¹⁸

2.7 Differential Thermal Analysis

Differential Thermal Analysis of Squibb House Standard #48101-001 showed an endotherm at 237°C. (Instrument: DuPont)¹

2.8 Thermogravimetric Analysis

Thermogravimetric analysis of Squibb House Standard #48101-001 gave no weight loss up to 150°C (Instrument: DuPont)¹

2.9 Solubility

Fluphenazine hydrochloride is hygroscopic and extremely soluble in water (70% w/v at 25°C pH 2.4).

Solubility in ethanol: 15% w/v at 25°C.

Less than 2% (w/v) in ether, acetone, benzene, ligroin.¹¹

Insoluble in chloroform and ether.⁶⁷

2.10 Crystal Properties

For forensic identification, a picture of fluphenazine dihydrochloride crystals from 95% ethanol has been published with those of other phenothiazines.¹⁹

An x-ray powder diffraction pattern of fluphenazine dihydrochloride is presented in

table 1.²⁰

Slightly different d-values (18.2; 9.07; 8.71; 6.07; 5.03; 4.85; 4.32; 4.02; 3.75)⁷ and (17.20; 11.50; 8.40; 5.90; 5.50; 5.40; 4.70; 4.50; 4.20; 4.10; 4.00; 3.90; 3.75; 3.60; 3.35; 3.20; 3.10; 2.85; 2.80; 2.65; 2.55; 2.45; 2.40; 2.22; 2.15)⁷⁷ were also reported.

3. SYNTHESIS

Synthetic pathways to fluphenazine (I) are shown in figure 6. The synthesis usually involves the addition of the propylpiperazine ethanol side chain to the 3-trifluoromethyl-phenothiazine nucleus (II). The following routes to (I) have been reported:

(1) Direct addition of 4(3-chloropropyl)-1-piperazine ethanol to (II) to yield (I).²¹

(2) Formation of intermediate 10(3-chloropropyl)-3 trifluoromethyl phenothiazine (III) under a variety of conditions, and condensation of (III) with piperazine ethanol to (I).^{12,16,17}

(3) Condensation of 3(2-trifluoromethyl-10-phenothiazinyl) propylpiperazine (IV) with ethylene oxide¹⁵, with β -bromo-ethyl acetate via (VI)^{13,14}, or with 2-bromo ethanol.⁶ This last method has also been used to prepare (I), labeled with ¹⁴C in the two ethanol carbons.⁶

(4) Reduction of 4(β -(10-phenothiazinyl) thio-propionyl) piperazine ethanol with lithium aluminum hydride to (I).²²

4. STABILITY - DEGRADATION

Fluphenazine hydrochloride is hygroscopic. When moisture is excluded, the crystalline material is stable for at least 6 months at temperatures as high as 50°C, both under air and nitrogen.¹¹ The compound is photosensitive, since the surface of crystals exposed to incandescent light for periods of more than 4 weeks discolored to

2.10 Crystal Properties (Continued)

TABLE I
X-ray Powder Diffraction pattern of
Squibb House Standard #48101-001

$d(\text{\AA})^*$	Rel. Intensity**	Curve No.
17.65 \pm 0.05	0.26	357
9.80	0.12	
8.90	0.58 (5)	
8.70	<u>0.26</u>	
6.02	0.42	
5.56	0.36	
5.46	0.24	
5.36	0.14	
5.15	0.31	
4.83 \pm 0.02	0.47	
4.75	0.59 (4)	
4.49	<u>0.71</u> (2)	
4.31	<u>0.48</u>	
4.23	0.22	
4.12	0.12	
4.00	<u>0.63</u> (3)	
3.90	<u>1.00</u> (1)	
3.72	<u>0.45</u>	
3.69	0.47	
3.57	0.32	
3.40	0.21	
3.27	0.16	
3.23	0.24	
3.11	0.42	
3.06	0.19	
3.00	0.22	
2.93	0.12	
2.87	0.15	
2.72	0.16	
2.67	0.16	
2.54	0.14	
2.42	0.15	
2.34	0.15	
2.27	0.14	
2.02	0.15	

* $d = (\text{interplanar distance}) \frac{n \lambda}{2 \sin \theta}$

$\lambda = 1.539 \text{ \AA}$

Radiation: $k\alpha_1$ and $k\alpha_2$ copper

** Based on highest intensity of 1.00
Instrument: Phillips

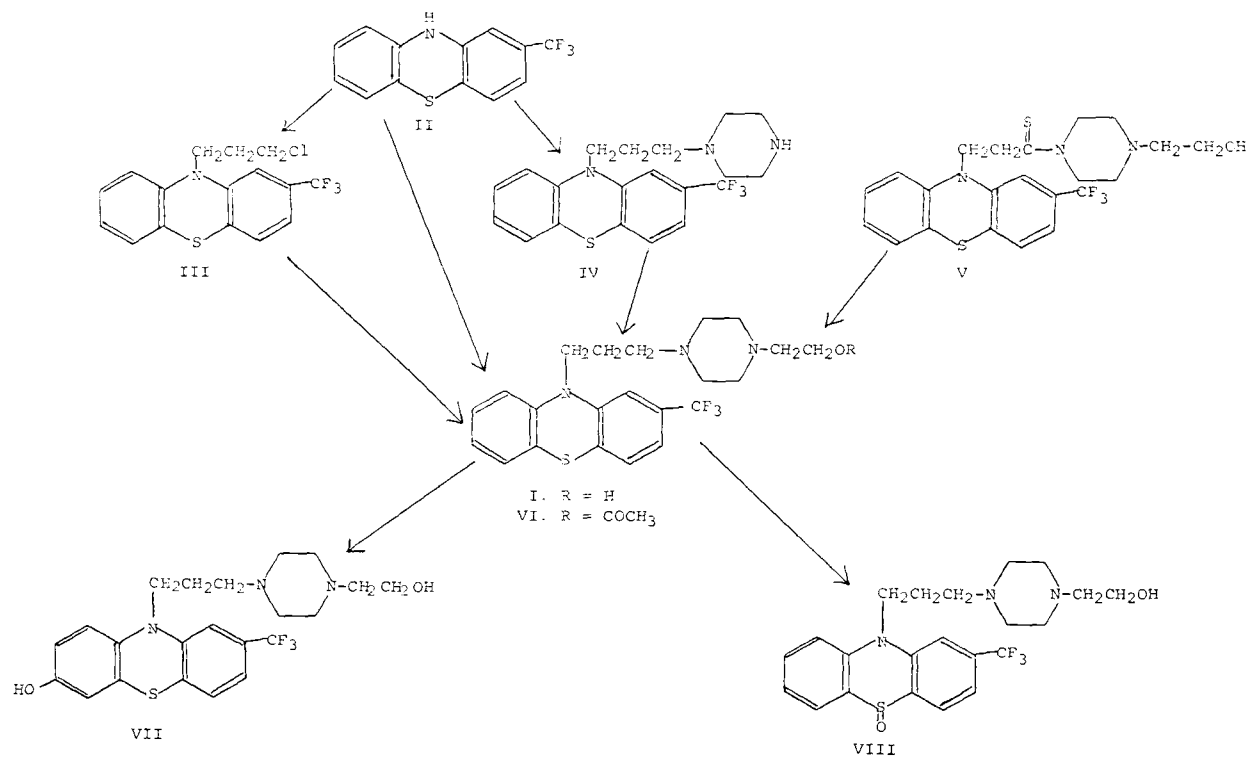


Fig. 6 Synthetic Pathways.

light tan. In aqueous solution, fluphenazine hydrochloride degrades when exposed to light. Solutions at 0.01% in ethanol, water, 0.1N HCl and 0.5N H_2SO_4 were kept in the dark or exposed to fluorescent ceiling light at room temperature (see section 2.3). After 17 days in the dark, all solutions retained their U.V. absorbance in the 255-265 $\text{m}\mu$ region. Only the 0.5N H_2SO_4 solution showed a slight decline in absorbance. In the light-exposed samples, only the ethanolic solution remained unchanged, while in the remaining solutions discoloration and the appearance of multiple bands at 240 and 255 $\text{m}\mu$ were noted.⁶⁴ Exposing a 0.05% fluphenazine hydrochloride in 0.1N HCl solution in pyrex glass to sunlight caused a 40% drop of absorbance in 2 hours.²³ A study of the pH dependency of the light instability showed a similar decline in absorbance from pH 0.3 to 9, whereas above pH 11 the degradation was retarded.²⁴ Again, it was found that solvents such as methanol, ethanol, dimethylformamide, ethylene glycol, and ether prevent or retard degradation.

The light-catalyzed degradation is not prevented by exclusion of oxygen²⁵ and proceeds through formation of the free radical (cf 26). The radical can also be formed by an oxidizing agent, such as ceric ammonium sulfate.²⁶ In phenothiazines generally, the semiquinone radical undergoes disproportionation. The products of oxidation are the sulfoxide or the 3(or 7)-hydroxy-derivatives. However, formation of the latter is severely inhibited in 10-substituted phenothiazines.²⁶ The second-order decay rate constant of the semiquinone radical of fluphenazine was found to be 1100 (l/mole/min), as measured by electron spin resonance.²⁶ The sulfoxide content of several commercial batches of fluphenazine was determined to be around 0.1% by

a spectrofluorometric method (excitation wavelength 350 mμ; fluorescence wavelength 400 mμ; see also section 6.4).²⁷ The sulfoxide content can also be measured by a thin-layer method (see also section 6.72).²⁸ So far, there is no evidence for the degradative formation of 7-hydroxyfluphenazine which has been identified as a metabolic product (see section 5).

5. DRUG METABOLISM

Acetylfluphenazine (VI, Figure 6) was hydrolyzed enzymatically to fluphenazine (I) in vitro by homogenates of small intestinal mucosa, liver, and brain from either the rat or the monkey.²⁹ (See also section 7). When fluphenazine was incubated with a "microsomal and soluble" fraction of rat livers, hydroxylation on the phenothiazine ring was observed.³⁰ The sulfoxide was not detected in the extract, and it seems unlikely that biological sulfoxidation precedes hydroxylation in these systems.³⁰ The biological disposition and metabolic fate of fluphenazine-¹⁴C in the dog and rhesus monkey was studied.⁷⁶

When dogs were treated with fluphenazine-¹⁴C, 7-hydroxyfluphenazine (VII, Figure 6) was isolated from the urine and identified by synthesis.³¹ In human blood and urine small amounts of fluphenazine and its sulfoxide, both in free and conjugated form were identified.⁷⁹ Tissue distribution and excretion were studied in the rabbit.⁷³

6. METHODS OF ANALYSIS

6.1 Elemental Analysis

Element	% Calc.	Ref.	% Found		
			11	12	15
C	51.76	51.72	51.73	51.68	
H	5.53	5.62	5.86	5.42	
F	11.17	-	-	-	
N	8.23	8.04	8.00	-	
O	3.13	-	-	-	
S	6.28	6.05	-	-	
Cl	13.89	13.66	-	-	

6.2 Nonaqueous Titration

Nonaqueous titration in glacial acetic acid with 0.01N perchloric acid and Quinaldine Red or crystal violet indicator gave a neutralization equivalent to 259 (calc. 255.22).³²

6.3 Polarographic Analysis

The half-wave potential in alcoholic sulfuric acid solution was found to be 0.714 (volt vs. normal calomel electrode), comparable to that of other phenothiazines.⁸

6.4 Spectrofluorometric Analysis

Fluphenazine hydrochloride, like other phenothiazine drugs, exhibits fluorescence. This phenomenon was first explored in 0.2N sulfuric acid.³³ The fluorescence peak was found in the range of 450 to 475 mμ, shifting to slightly lower wavelength upon oxidation with potassium permanganate. As little as 0.01 to 0.05 μg of the pure substance per ml. of 0.2N sulfuric acid could be detected. Due to interference by other substances, the sensitivity in biological fluids was lower (about 0.8 μg/ml fluid). This method is superior to determination by U.V., where about 4 μg/ml are required.

In 50% acetic acid, the following data were obtained, without or with oxidation with 30% hydrogen peroxide³⁴:

<u>Activation maximum, mμ</u>		<u>Fluorescence maximum, mμ</u>	
Unoxidized	Oxidized	Unoxidized	Oxidized
Ref 34 330	345	460	380
Ref 35 -	350	-	410

In the unoxidized form, a sharp increase in fluorescence intensity occurred above pH 7; in the oxidized form, intensity was about equal from

pH 2 to pH 12 and fell precipitously at pH 12. In the oxidized form, as little as 0.5 $\mu\text{g}/\text{ml}$ could be determined quantitatively. Fluphenazine was relatively less fluorescent than some other phenothiazines.

In concentrated sulfuric acid, the activation maximum was found at 325 $\text{m}\mu$ and the fluorescence maximum at 500 $\text{m}\mu$.³⁶

The instrument used in all investigations was the Aminco-Bowman Spectrofluorometer.

6.5 Colorimetric Analysis - Color Tests for Identification

The general method of Ryan³⁷ to quantitate unoxidized phenothiazine derivatives by color complex formation with palladium chloride (500-550 $\text{m}\mu$ maxima) has also been adopted for fluphenazine hydrochloride (see N.F.XIII) and can be used for stability measurements.

Like other phenothiazines, fluphenazine hydrochloride reacts with ceric sulfate, first by forming a red-colored semiquinone free radical (420 $\text{m}\mu$), followed by formation of the colorless sulfoxide derivative. This reaction has been made the basis of a photometric titration.³⁸

The following color tests have been reported. (For additional tests, see also sections 6.71 and 6.72).

FOR COLOR TESTS SEE TABLE II

6.6 Electrophoresis

Electrophoresis was carried out on fluphenazine hydrochloride and several other phenothiazine tranquilizers in various buffers proposed by Werrum.⁴¹ A Gordon-Misco apparatus, Whatman 3MM paper 30 cm in width, and a potential difference of 500 to 800 v were used. Detection of spots was carried out with a 40% sulfuric acid

TABLE II

<u>Reagent</u>	<u>Color</u>	<u>Ref</u>
0.001N Mercuric nitrate in hydrochloric acid	pink-violet	39
0.5% aq. ammonium molybdate	blue-pink-violet	40
0.5% aq. ammonium vanadate	grey-yellow	40
37% formaldehyde in conc. sulfuric acid (1:20)	light red	40
0.5% aq. selenious acid	grey-green	40
1% aq. sodium tungstate	purple	40
0.5% titanium dioxide in conc. sulfuric acid	orange	40
Fuming nitric acid, then ethanolic potassium hydroxide (3%)	yellow-green	40
10% aq. sucrose	grey-yellow	40
Conc. sulfuric acid	light brown	40
p-dimethylaminobenzaldehyde 10% in glacial acetic acid	light brown	40
1% cobalt acetate; 10% isopropylamine in acetone	light blue	18
10% aq. chloramine T	light yellow	18
1% aq. palladium chloride acidified with hydrochloric acid	orange	18
Nitric acid	orange-brown	18
Conc. sulfuric acid, 6% uranium nitrate in 95% ethanol (3:17)	light orange	18
0.5% ammonium vanadate in conc. sulfuric acid	yellow brown	18
4% aq. silver nitrate solution acidified with 0.1N sulfuric acid	cream white	18
2% ammonium ferrous sulfate in conc. sulfuric acid	-	75

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spray (orange color) or fluorescence under UV light.⁴²

For fluphenazine hydrochloride, anodic migration (cm) after 40 to 60 minutes was:

pH:	3.3	4.7	6.0	7.2	8.0	9.3
Migration:	6.8	3.7	4.3	4.5	2.7	1.2

6.7 Chromatographic Analysis

6.71 Paper Chromatographic Analysis

The following chromatographic systems have been reported:

<u>Solvent Systems:</u>	<u>R_f</u>	<u>Ref</u>
1N Sodium formate	0.23	42
1N Sodium formate 90/n-Propanol 10	0.32	42
1N Sodium formate 90/1N Ammonia 10	0.19	42
1N Sodium formate 97/Formic acid 3	0.42	42
1N Sodium acetate	0.17	42
1N Sodium acetate 90/n-Propanol 10	0.48	42
10% Sodium chloride 92/n-Propanol 8	0.45	42
Formamide + Ammonium formate/Benzene	0.71	43
Formamide + Ammonium formate/Cyclohexane-benzene (9:1)	0.22	43
Petroleum (b.p. 195-220°)/Ethanol-Ammonia-water (60:2:38)	0.59	43

<u>Detection systems:</u>	<u>Color</u>	<u>Ref</u>
40% H ₂ SO ₄ spray	orange	42,43
Fluorescence under UV light	bluish yellow	42,43
Dragendorff-reagent	orange	43
Cerium sulfate in 2N sulfuric acid	red	43
1% gold (III) chloride	red	43
1% dimethylaminobenzaldehyde in ethanol:chloroform (95:5)	red	43

A quantitative paper chromatographic method⁶² can be used to measure the purity of

fluphenazine, following published procedures⁶³. The paper is impregnated with castor oil (USP) in ether. Developing solvent is methanol-water 85:15. R_f values of fluphenazine base approx. 0.79, fluphenazine hydrochloride 0.83. The zones are eluted from the chromatogram with 95% ethanol and the absorbances of the eluates are determined at 261 m μ in a spectrophotometer.

6.72 Thin-Layer Chromatographic Analysis

The following thin-layer chromatographic systems have been reported:

FOR ABOVE SEE TABLE III

6.73 Column (Ion-exchange) Chromatographic Analysis

Ion-exchange analysis of fluphenazine hydrochloride and other phenothiazine tranquilizers on a column of Dowex sulfuric acid resin 50W-X8 was possible, but not too practical for analysis of tablets.⁵⁶

6.74 Gas-Liquid Chromatographic Analysis

Fluphenazine was not eluted after 90 minutes at 270^o, using a 210 SE-30 silicone polymer on a 80-100 mesh Gas-chrom S diatomaceous earth column⁵⁷; with a 3% SE-30 on 80-100 mesh Gas-chrom Q at 250^oC, it eluted in 4.9 minutes.²⁸ Fluphenazine was also well separated from other tranquilizers on a 120 mesh silanized Gas-chrom P column coated with 1% Hi-Eff-8B (cyclohexane-dimethanol succinate) at a temperature of 220^oC and a retention time of 5 minutes.⁵⁸ The identification of fluphenazine and other phenothiazine tranquilizers by gas chromatography of their pyrolysis products has also been reported.⁵⁹

TABLE III

Adsorbent	Solvent System	R _f	Ref
Silica Gel	tert. Butyl alcohol 90/1N Ammonia 10	0.37	42
"	n-Propanol 88/1N Ammonia 12	0.56	"
"	Ether, sat. with water	0.09	"
"	70% Methanol	0.68	"
"	85% n-Propanol	0.27	"
"	n-Butanol sat. with 1N Ammonia	0.57	"
"	Benzene-Dioxane-aq. Ammonia (60:35:5)	0.34	52
"	Ethanol-Acetic acid-water (50:30:20)	0.58	"
"	Methanol-butanol (60:40)	0.68	"
"	Cyclohexane-Diethylamine (9:1)	0.05	53
Cellulose	5% aq. Ammonium sulfate sat. with Isobutanol	0.03	54
Silica Gel	Cyclohexane-methanol (50:50 and 25:75)	0.36	44
"	Chloroform-acetone (50:50)	0.03	44
"	Chloroform-methanol (50:50)	0.59	51, 44, 50
"	Chloroform-methanol (90:10)	0.44	45
"	Chloroform-acetone-methanol (50:25:25)	0.42	44
"	Chloroform-acetone-methanol (25:50:25)	0.32	44
"	Chloroform-acetone-methanol-ammonia (50:25:25:1)	0.54	44
"	Acetone	0.06	44
"	Acetone	0.16	45
"	Acetone-ammonia (100:1)	0.33	44, 51

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TABLE III Cont'd.

Adsorbent	Solvent System	R _f	Ref
Silica Gel	Acetone-ammonia (95:5)	0.54	44
"	Acetone-methanol (50:50)	0.38	"
"	Acetone-methanol-ammonia (50:50:1)	0.56	"
"	Methanol	0.34	"
"	Methanol-ammonia (98:2)	0.54	"
"	Methanol-ammonia (98:2)	0.67	45
"	Chloroform-methanol-ammonia (80:20:1)	0.68	30
"	Chloroform-ethanol-ammonia (80:20:1)	0.76	23
"	Acetone-benzene-ammonia (30:70:5)	--	46
"	Butanol-pyridine-water (1:2:1)	0.52	47
"	Cyclohexane-benzene-diethylamine (75:15:10)	0.06	48
"*	Cyclohexane-benzene-diethylamine (75:15:20)	0.18	45
"*	Methanol	0.60	48
"*	Acetone	0.25	"
"*	Benzene-ethanol-ammonia (95:15:5)	0.30	45
"**	Methanol	0.15	48
"**	95% ethanol	0.06	"
Silica Gel, Plain	Chloroform + cyclohexane-diethylamine (50:40:10)	0.24	49
Chromagram, Plain	Chloroform + cyclohexane-diethylamine	0.48	"

TABLE III Cont'd

<u>Adsorption</u>	<u>Solvent System</u>	<u>R_f</u>	<u>Ref</u>
Chromagram			
+ fluor indicator	Chloroform + cyclohexane-diethylamine	0.40	49
Silica Gel	Chloroform-hexane (1:1)	0.48	50
"	15% aq.ammonium acetate-methanol (20:80)	0.75	28***

* prepared with 0.1M KOH

** prepared with 0.1M NaHSO₃

*** In this system, fluphenazine-sulfoxide had an R_f of 0.59

The following detection systems have been used:

<u>System</u>	<u>Color</u>	<u>Reaction</u>	<u>Ref</u>
Fluorescence under U.V. lamp	-		42,44,51,30,28,50
40% sulfuric acid spray	orange		42,46,47
50% aq.sulfuric acid in ethanol (4:1)	orange-red		47,52
Potassium iodoplatinate	violet		52,54,44,51,49,28
5% ferric chloride, 20% perchloric acid, 50% nitric acid (5:45:50) (FPN;Forrest reagent)	(flesh or pale pink)		44,45,51,53,54,48
Iodine vapors	brown		54,44,48
1% potassium permanganate			51
Dragendorff reagent	brown		51,48

Detection systems used Cont'd.

<u>System</u>	<u>Color</u> <u>Reaction</u>	<u>Ref</u>
5% p-dimethylaminobenzaldehyde in 18N sulfuric acid	pink or orange	45,47,51
Furfural reagent	cameo	51,45
1% cerium sulfate in 2N sulfuric acid	red	47
1% gold (III) -chloride	red	47
Folin-Ciocalteu reagent	cameo	45
1% ammonium vanadate in 10 ml. conc. sulfuric acid	flesh	45,48
5% cinnamic aldehyde and 5% HCl in ethanol	cameo	45
p-benzoquinone in dichloroethane	-	55

7. Countercurrent Separation

Fluphenazine was separated from acetyl fluphenazine (VI, figure 6) by countercurrent distribution (see also section 5) across 25 tubes in a solvent system of 0.1M acetate buffer, pH 3.82, and 1.5% isoamyl alcohol in heptane.²⁹

8. Identification and Determination in Body Fluids and Tissues

Many of the references cited in previous sections concern methods devised to identify and determine fluphenazine hydrochloride and other phenothiazines for pharmacological, toxicological, and forensic purposes (cf. 60). These methods can be classified as follows:

Color reactions	43,45,55,39,18,40,74,75
Separation schemes	61,43,45,51,74,79
Spectrofluorometry	33,34,35,36,68
Electrophoresis	42
Paper chromatography	43,43
Thin-layer chromatography	(28,42-49, 51-54, 65,74, 78,79)
Gas-liquid chromatography	28,57,58
Solubility	65
X-ray diffraction bands	7
Microcrystalline identification	19,72

9. Miscellaneous

The absorption of fluphenazine dihydrochloride and other phenothiazine derivatives by solid adsorbants, such as kaolin, talc, and charcoal, has been studied by Sorby et al.¹⁰ The volumetric determination of fluphenazine by stoichiometric combination with anionic surface-active agents, such as sodium lauryl sulfate and sodium dioctyl sulfosuccinate, has been

described.⁶⁶ The surface tension-lowering activity of fluphenazine and other tranquilizers has been studied.⁷⁰ Ion-pair extraction has been described.⁷¹

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ISOCARBOXAZID

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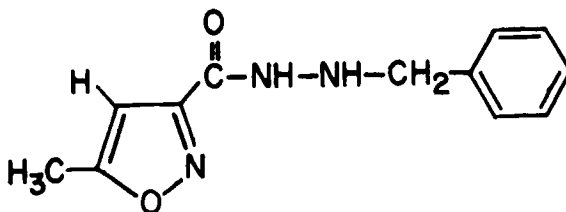
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1. Description1.1 Name, Formula, Molecular Weight

Isocarboxazid is 5-methyl-3-isoxazolecarboxylic acid 2-benzylhydrazide.


 $C_{12}H_{13}N_3O_2$

Molecular Weight: 231.26

1.2 Appearance, Color, Odor

Isocarboxazid occurs as a white crytalline powder. It has a slight, characteristic odor.

2. Physical Properties2.1 Infrared Spectrum (IR)

The IR spectrum of bulk reference standard isocarboxazid was obtained from a pellet made by dispersing 1.1 mg of isocarboxazid in 300 mg of KBr (1). This spectrum, shown in Figure 1, was measured on a Perkin Elmer 621 Spectrophotometer. A list of the assignments made for some of the characteristic bands is given in Table I (1).

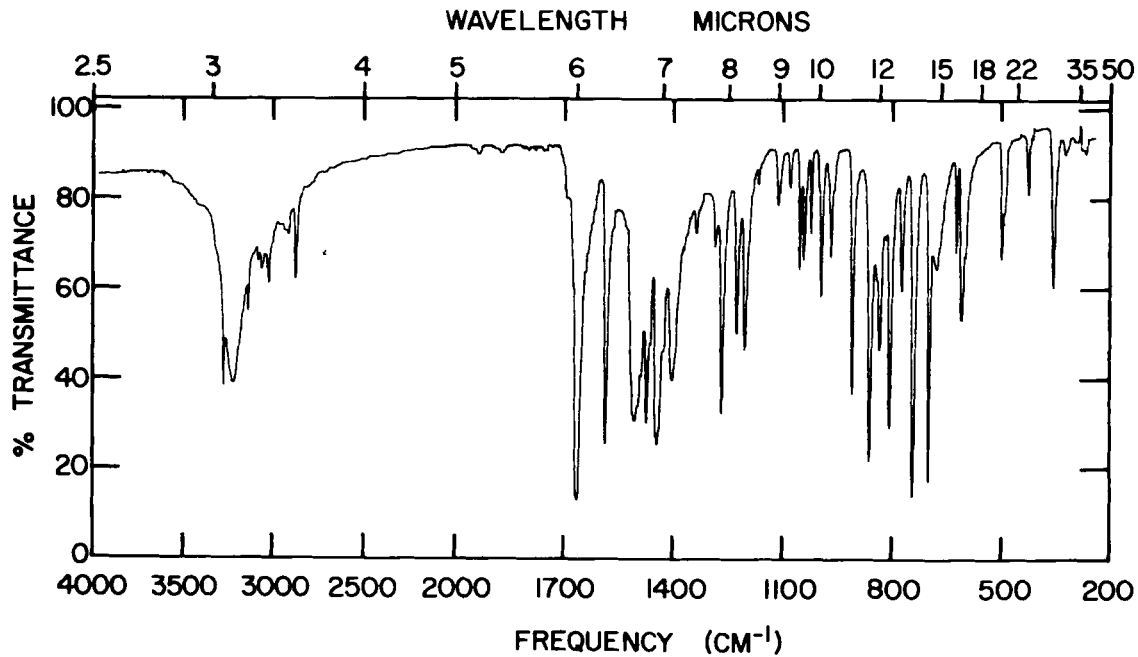
TABLE I

Characteristic Bands in the IR Spectrum of Isocarboxazid

<u>Wavelength (cm⁻¹)</u>	<u>Characteristic of</u>
3269 and 3212	NH stretching vibrations
2000 to 1700	Overtone bands of singly substituted benzene
1669	C=O stretch
1593 and 1453	C=C stretch of aromatics
750 and 704	Deformations of 5 adjacent hydrogens on benzene ring

Figure 1

Infrared Spectrum of Isocarbrazid



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2.2 Nuclear Magnetic Resonance Spectrum (NMR)

The NMR spectrum shown in Figure 2 was obtained by dissolving 57.3 mg of reference standard isocarboxazid in 0.5 ml of CDCl_3 containing tetramethylsilane as the internal reference. The spectral assignments are shown in Table II (2).

TABLE II

NMR Spectral Assignments for Isocarboxazid

Type Proton	No. of Each Proton	Chemical Shift (ppm)	Multiplicity
$-\text{CH}_3$	3	2.45	S
$-\text{CH}_2-$	2	4.07	S
$-\text{NH}-$	1	4.85	S(b)
$=\text{CH}-$	1	6.47	S
$-\text{C}_6\text{H}_5$	5	7.39	S
$-\text{CONH}-$	1	9.75	S(b)

S= sharp singlet; S(b)= broad singlet

2.3 Ultraviolet Spectrum (UV)

The UV spectrum of isocarboxazid in 2-propanol in the region 350 to 210 nm exhibits no maxima or minima but a shoulder occurs at 228-229 nm ($\epsilon=6.6 \times 10^3$). The spectrum presented in Figure 3 was obtained from a reference standard solution of isocarboxazid at a concentration of 1.014 mg per 100 ml of 2-propanol (3).

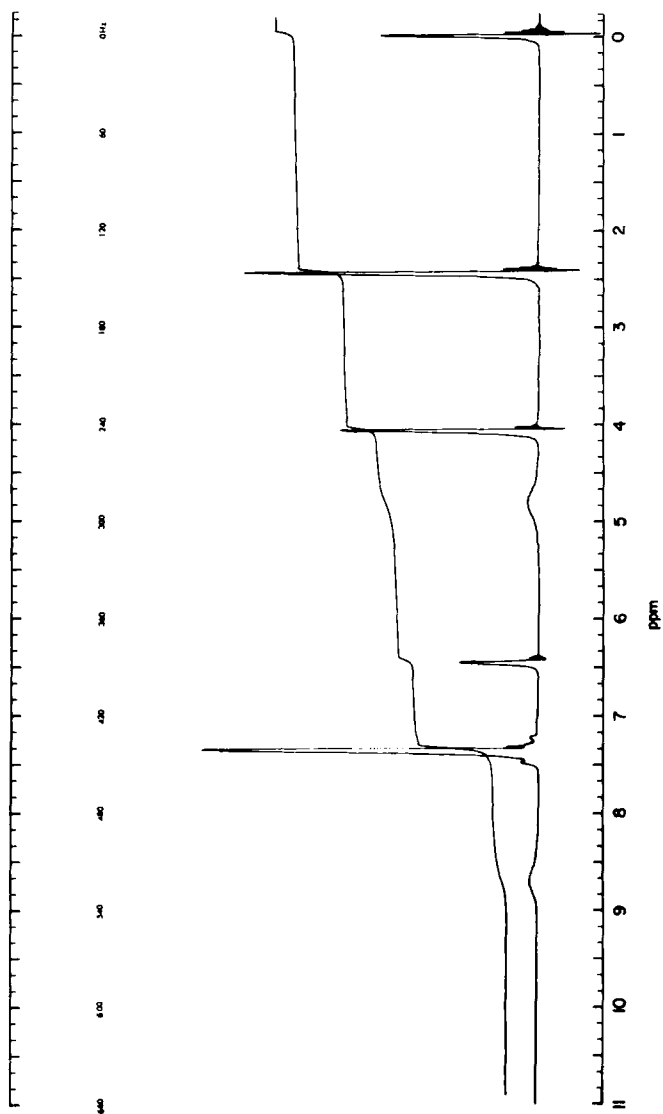
2.4 Fluorescence Spectrum

Solutions of isocarboxazid in methanol, 0.1N HCl, and 0.1N NaOH displayed no fluorescence when the excitation energy used was white light (4).

2.5 Mass Spectrum

The mass spectrum of reference standard isocarboxazid shown in Figure 4 was obtained using a CEC 21-110 spectrometer with an ionizing energy of 70 eV (5). Table III lists the diagnostic peaks appearing in the low resolution spectrum.

Figure 2
NMR Spectrum of Isocarboxazid



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Figure 3

Ultraviolet Spectrum of Isocarboxazid

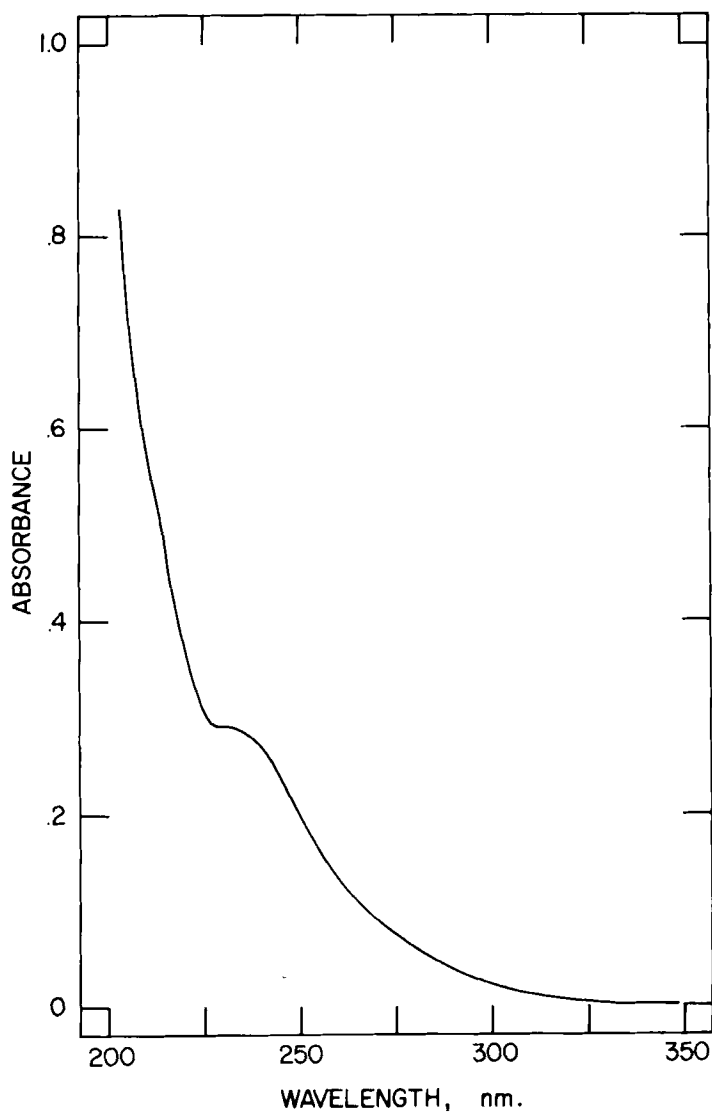
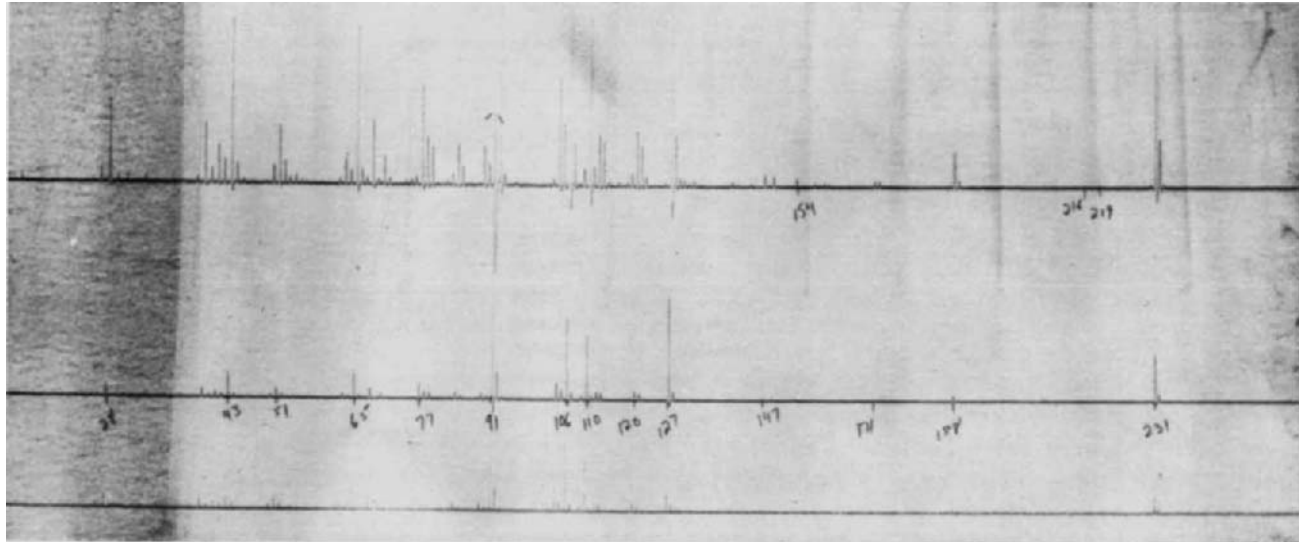


Figure 4



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TABLE III

<u>Mass (m/e)</u>	<u>Intensity</u>	<u>Fragment Ions</u>
231	Medium	Molecular ion (M)
216	Very weak	M-CH ₃
171	Very weak	M-C ₂ H ₆ NO
154	Very weak	M-C ₆ H ₅
147	Weak	C ₈ H ₇ N ₂ O
127	Strong	C ₅ H ₇ N ₂ O ₂
110	Medium	C ₅ H ₄ NO ₂
106	Medium	C ₆ H ₅ CH ₂ NH
91	Very strong	C ₆ H ₅ CH ₂

2.6 Optical Rotation

Isocarboxazid exhibits no optical activity.

2.7 Melting Range

The melting range is dependent on the heating rate. Using the Class I melting procedure outlined in NF XIII, isocarboxazid melts between 105° and 108°C (6).

2.8 Differential Scanning Calorimetry (DSC)

A Perkin Elmer DSC-1B Calorimeter was used to obtain the DSC curve for reference standard isocarboxazid shown in Figure 5. With a temperature program of 10°C/min., a melting endotherm was observed starting at 104.7°C and a broad exotherm due to decomposition starting about 150°C. The ΔH_f calculated from the melting endotherm was 7.4 kcal/mole (7).

2.9 Thermogravimetric Analysis (TGA)

The TGA performed on reference standard isocarboxazid exhibited no loss of weight when heated to 120°C at a rate of 10°C/min. (7).

2.10 Solubility

The solubility data obtained at 25°C for reference standard isocarboxazid is listed in Table IV (8).

Figure 5

DSC Curve of Isocarboxazid

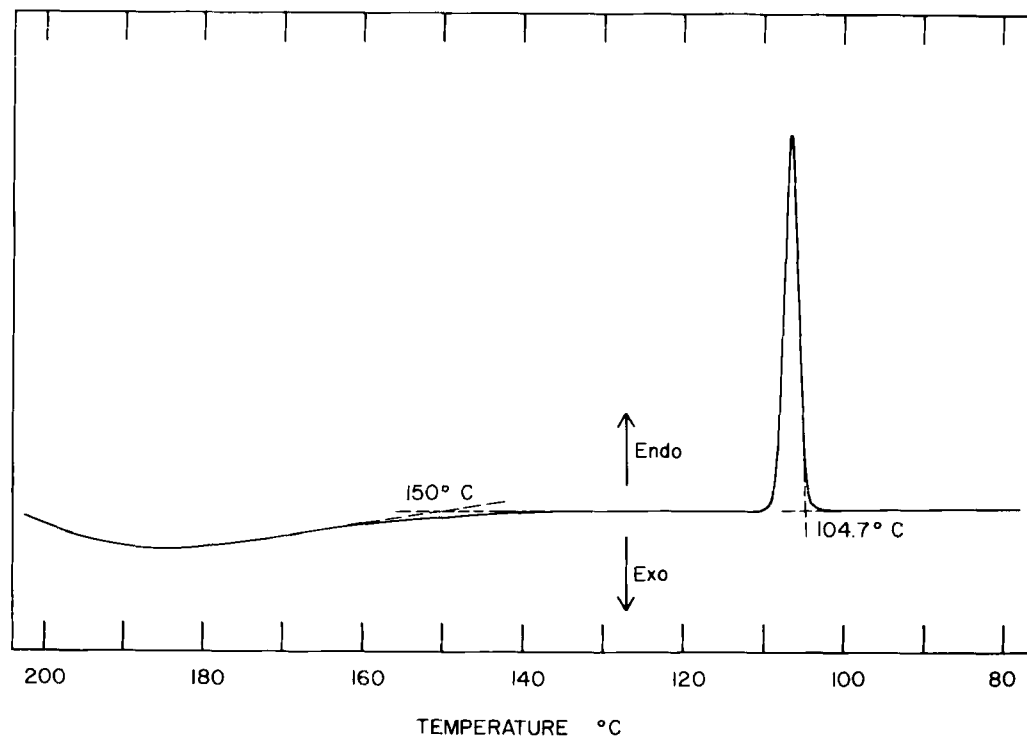


TABLE IV

Isocarboxazid - Solubility

<u>Solvent</u>	<u>Solubility (mg/ml)</u>
3A alcohol	39.6
benzene	44.8
chloroform	348.6
95% ethanol	46.8
ethyl ether	16.5
methanol	95.1
petroleum ether (30°-60°)	0.2
2-propanol	16.5
water	0.8

2.11 X-ray Crystal Properties

The x-ray powder diffraction pattern of reference standard isocarboxazid is presented in Table V (9). The instrumental conditions are given below:

Instrumental Conditions:

General Electric Model XRD-6 Spectrogoniometer

Generator:	50 KV, 12-1/2 MA
Tube target:	Copper
Radiation:	Cu K α = 1.542 Å
Optics:	0.1° Detector slit
	M.R. Soller slit
	3° Beam slit
	0.0007" Ni filter
	4° take off angle
Goniometer:	Scan at 0.2° 2 θ per minute
Detector:	Amplifier gain - 16 coarse, 8.7 fine
	Sealed proportional counter tube and DC voltage at plateau, Pulse height selection E _L - 5 volts; E _u - out, Rate meter T.C. 4, 2000 C/S full scale
Recorder:	Chart speed 1"/5 minutes
Samples:	Prepared by grinding at room temperature

TABLE V

X-ray Diffraction Pattern of Isocarboxazid

2θ	$d^{\circ}A$	I/I_0^{**}
9.100	9.7177	41
12.440	7.1151	5
13.460	6.5781	9
14.860	5.9614	13
15.240	5.8136	9
17.640	5.0276	5
18.440	4.8113	4
19.040	4.6610	16
20.700	4.2908	44
21.260	4.1790	9
21.720	4.0916	7
23.240	3.8273	15
23.480	3.7887	18
23.920	3.7200	28
24.960	3.5673	100
25.780	3.4557	9
27.300	3.2666	3
28.660	3.1146	5
29.060	3.0726	6
29.680	3.0099	1
29.780	3.0000	2
30.160	2.9630	2
30.280	2.9516	3
32.480	2.7565	4
34.520	2.5981	7
34.800	2.5779	7
35.780	2.5095	2
36.540	2.4590	4
37.400	2.4044	1
37.940	2.3714	1
38.120	2.3606	1
38.760	2.3231	<1
39.340	2.2902	1
40.920	2.2053	3
41.420	2.1799	2
41.860	2.1580	3
42.320	2.1356	2
43.080	2.0996	1

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45.480	1.9943	1
45.880	1.9778	2
46.480	1.9537	2
46.860	1.9387	3
47.980	1.8960	3
49.140	1.8539	3
50.140	1.8193	3
51.680	1.7686	2

$$*d = (\text{interplanar distance}) \frac{n\lambda}{2 \sin \theta}$$

**I/I_o = relative intensity (based on highest intensity of 1.00)

2.12 Dissociation Constant

The apparent pK_a for isocarboxazid has been determined spectrophotometrically to be 10.4 (10).

3. Synthesis

Isocarboxazid may be prepared by the reaction scheme shown in Figure 6. Methyl 5-methyl-3-isoxazolecarboxylate is reacted with benzylhydrazine to form isocarboxazid (11).

4. Stability Degradation

Isocarboxazid is stable when present in crystalline form. When heated at 100°C in 0.1N HCl, water, and 0.1N NaOH, hydrolysis occurs. After one hour of heating, 7% of the isocarboxazid was hydrolyzed in the 0.1N HCl solution, 12% in the water solution, and 100% in the 0.1N NaOH solution (12).

5. Drug Metabolic Products

The predominant pathway of isocarboxazid metabolism in man is the cleavage and oxidation of the benzyl moiety to benzoic acid followed by a conjugation with glycine to give hippuric acid (13). A second metabolic mechanism is the hydrolytic cleavage of isocarboxazid forming benzyl hydrazine. This latter pathway is more predominant in guinea pigs than in man (14). Figure 7 outlines these metabolic pathways.

6. Methods of Analysis

6.1 Elemental Analysis

Figure 6
Synthesis of Isocarboxazid

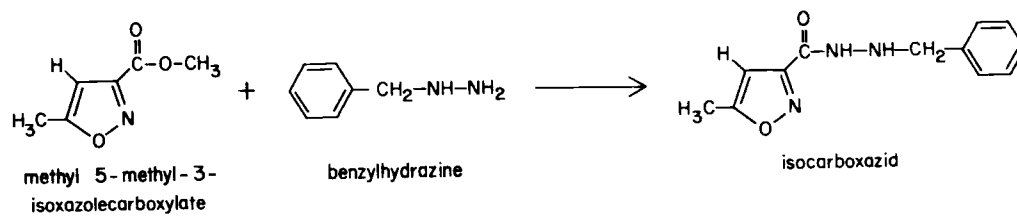
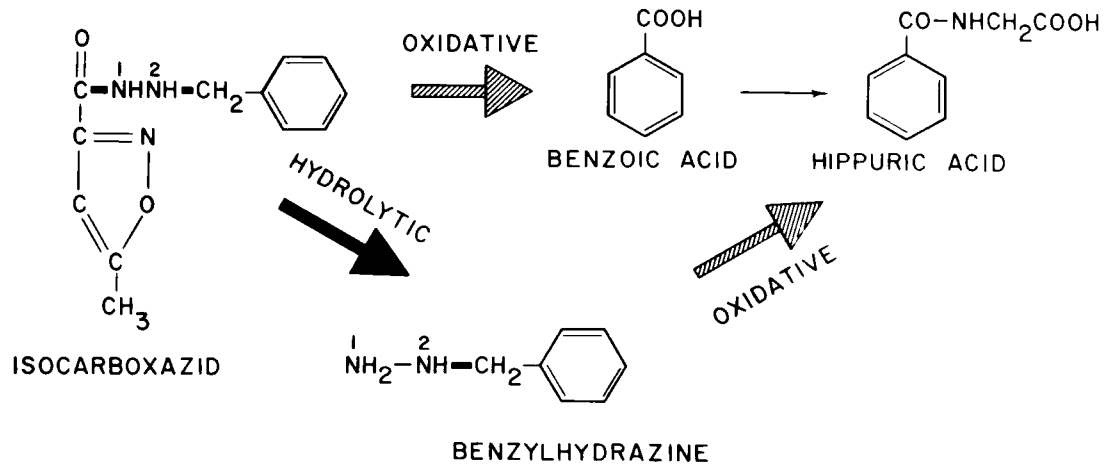


Figure 7

Metabolic Products of Isocarboxazid (13)



The results from an elemental analysis of reference standard isocarboxazid are presented in Table IV (15).

TABLE VI

Elemental Analysis of Isocarboxazid

<u>Element</u>	<u>% Theory</u>	<u>% Found</u>
C	62.33	62.22
H	5.67	5.66
N	18.17	18.20

6.2 Phase Solubility Analysis

Phase solubility analysis has been carried out for isocarboxazid using 2-propanol as the solvent. An example is presented in Figure 8 for reference standard isocarboxazid along with the conditions under which the analysis was performed (8).

6.3 Thin Layer Chromatographic Analysis (TLC)

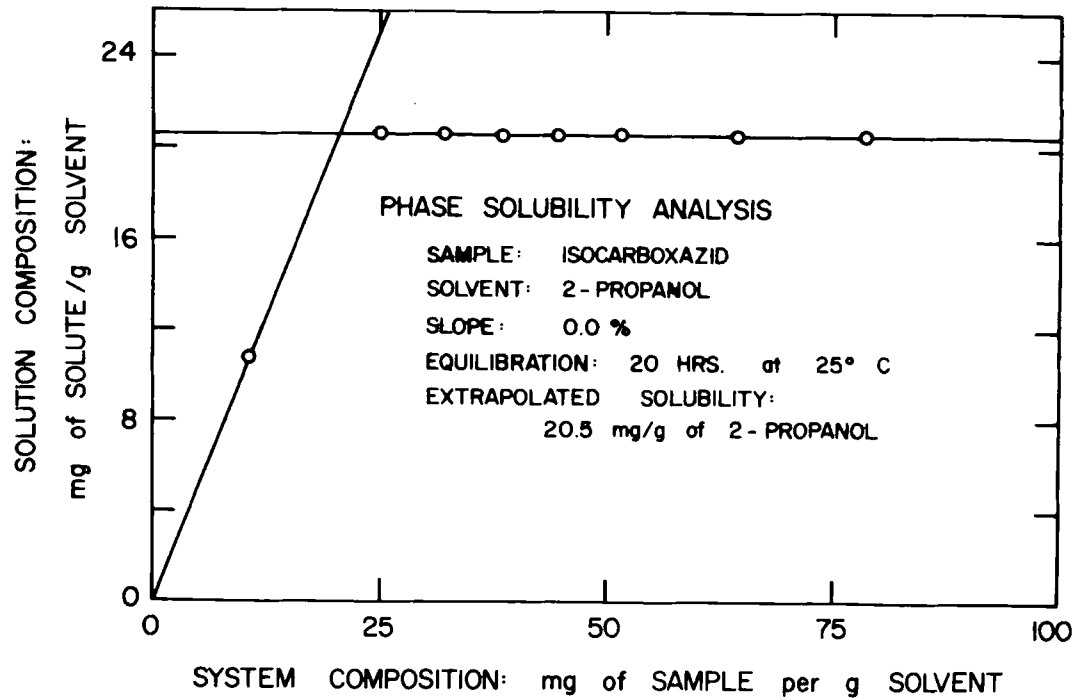
The following TLC procedure is useful for separating methyl 5-methyl-3-isoxazolecarboxylate and 1-benzyl-3-methyl-5-aminopyrazole from isocarboxazid (6). Using silica gel GF plates and ethyl acetate: n-heptane (60:40) as the solvent system, 1.0 mg of the sample in methanol is spotted on the plate and subjected to ascending chromatography. After the solvent front has ascended at least 12 cm, the plate is air dried and examined under shortwave ultraviolet light. If any methyl 5-methyl-3-isoxazolecarboxylate is present it will appear as a dark spot at about R_f 0.85. Next spray the plate with a freshly prepared solution of 10% FeCl_3 :5% $\text{K}_3\text{Fe}(\text{CN})_6$ (1:1). If any 1-benzyl-3-methyl-5-aminopyrazole is present it will appear as a blue spot at about R_f 0.25. The isocarboxazid produces a blue spot at about R_f 0.6.

6.4 Colorimetric Analysis

6.41 Ammonium Molybdate Reaction

The isocarboxazid content in tablets may be determined by the following procedure (6). The tablets are finely ground, a portion equivalent to about 10 mg of isocarboxazid is weighed into a 50 ml volumetric flask and

Figure 8



ISOCARBOXAZID

diluted to volume with acetone. After thorough mixing, the solution is clarified by centrifuging and 5.0 ml is pipetted into a stoppered flask. To the flask are also added 1.0 ml of water, 50.0 ml of acetone, and 1.0 ml of ammonium molybdate reagent (1 gm of ammonium molybdate dissolved in 100 ml of dilute HCl). The flask is stoppered and the solution mixed and then allowed to stand 30 minutes with occasional swirling. The absorbance of this solution is determined at 420 nm against a blank. Reference standard isocarboxazid is prepared at approximately the same concentration and in a similar manner as above. The mg of isocarboxazid per tablet is calculated by the following formula:

$$\frac{(A_{\text{sample}})(\text{mg standard})(\text{Av. tablet wt. in mg})}{(A_{\text{standard}})(\text{mg sample})(5)}$$

where 5 is the dilution factor.

6.42 Tetrazolium Salt Reaction

The isocarboxazid content in blood may be determined in the range of 0.5 µg/ml of plasma by the following procedure. Add 1.5 ml of pH 7.3 phosphate buffer to 1 ml of blood and let stand for 10 minutes. Add 4 ml of butyl acetate:butanol (9:1) and 0.75 gm of Na₂SO₄: MgO powder (30:1). Shake for 30 minutes and then centrifuge until the phases separate. Pipet 3 ml of the organic phase into a test tube, add 0.1 ml of the tetrazolium reagent (0.125% 3,3'-dianisole-bis-4,4'-(diphenyl)-tetrazolium chloride in methanol) and 0.1 ml of 0.02 N KOH. Place in a bath of boiling water exactly one minute then cool rapidly by immersing in cold water. The isocarboxazid has reduced the tetrazolium salt to a blue formazan. Read the absorbance of the solution at 520 nm within 30 minutes. At the same time run the above procedure on a 1 ml blood blank and a 1 ml blood sample containing 5 µgm of reference standard isocarboxazid. Correct for the blank and use the reference standard to calculate an extinction coefficient which is used to calculate the unknowns (16,17).

6.5 Potentiometric Sodium Nitrite Titration

The potentiometric sodium nitrite titration as described in the NF XIII is the method of choice for the analysis of the isocarboxazid fine chemical (6). A sample

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of about 700 mg is accurately weighed and dissolved in 20 ml of glacial acetic acid. Following dissolution 20 ml of HCl and 40 ml of water is added and the solution cooled to room temperature. Titrate potentiometrically with 0.1N NaNO_2 solution using a calomel-platinum electrode system. Each ml of 0.1N NaNO_2 is equivalent to 23.13 of isocarboxazid.

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ISOPROPAMIDE

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Gerald D. Roberts, Edward White, V, and Peter P. Begosh*

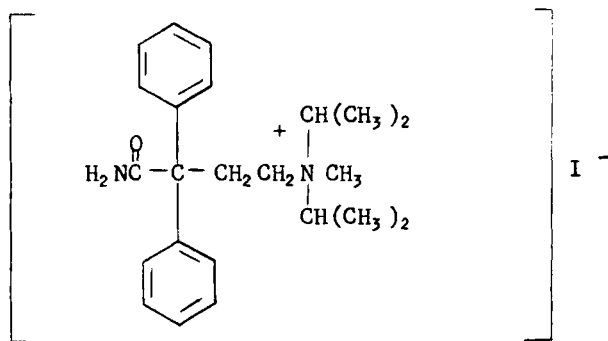
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7. Notes and References

1. Description

1.1 Name, Formula, Molecular Weight

Isopropamide is (3-Carbamoyl-3,3-diphenylpropyl)-diisopropylmethylammonium iodide. It is also known as Darbid; Priamid; Tyrimide; 2,2-diphenyl-4-diisopropylaminobutyrimide methiodide.



$\text{C}_{23}\text{H}_{33}\text{IN}_2\text{O}$

Mol. wt.: 480.443

1.2 Appearance, Color, Odor, Taste

A white to off-white (very pale yellow) crystalline or amorphous powder, odorless, with an extremely bitter taste.

2. Physical Properties

2.1 Infrared Spectrum

Figure 1 is the infrared spectrum of isopropamide (SK+F standard SJB-4406-226A) taken in a mineral oil dispersion from $4000 - 625 \text{ cm}^{-1}$ on a Perkin-Elmer Model 457. R. Warren assigns the following bands (cm^{-1}) to isopropamide:

3530 and 3475; free NH

3300; bonded NH_2

1665; amide $\text{C}=\text{O}$

1585 and 1490; aromatic $\text{C}=\text{C}$ and NH_2

770-710; aromatic CH out-of-plane deformation
mono-substituted phenyls

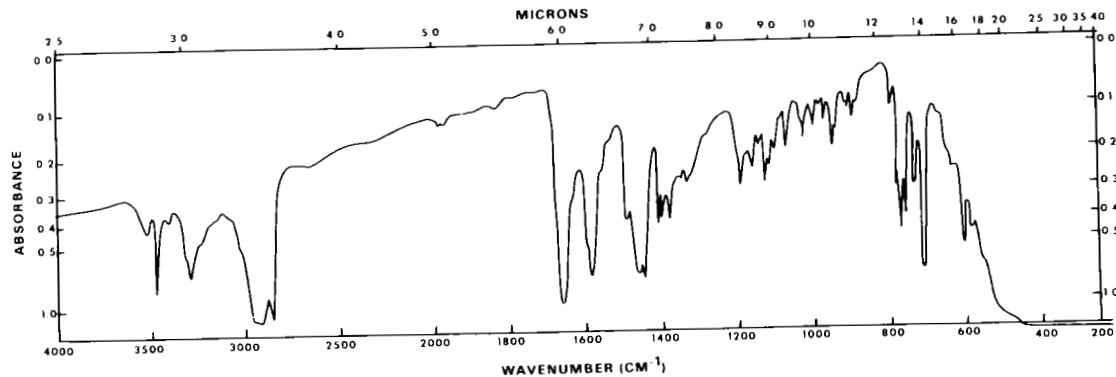
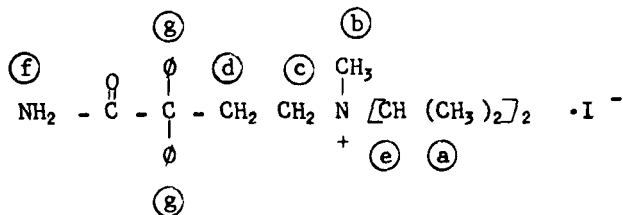


Figure 1. Isopropamide - SK+F standard SJB-4406-226A, mineral oil, dispersion, Instrument: Perkin-Elmer 457.

2.2 Nuclear Magnetic Resonance Spectrum

The NMR spectrum (Figure 2) was obtained in a deuterio chloroform solution of SK+F standard SJB-4406-226-A which contained about 100 mg/ml and tetramethylsilane as internal reference on a JEOL Model C 60 H. The following assignments (Hz) were made by R. Warren:



82; doublet, protons at (a)

177; protons at (b) (c) (d)

244.5 ; multiplet, protons at (e)

340 and 387; protons at (f)

447; protons at (g)

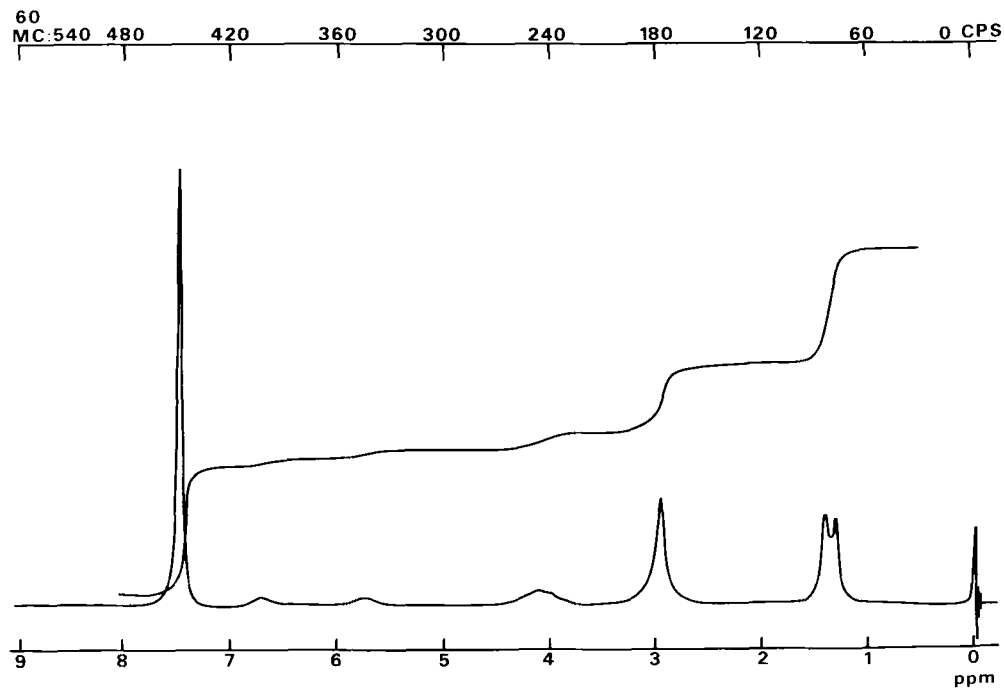


Figure 2. NMR Spectrum Isopropamide, SK+F standard SJB-4406-226A in deuteriochloroform tetramethylsilane internal reference, Instrument: JEOL C 60 H.

2.3 Ultraviolet Spectra

The ultraviolet absorption spectrum of isopropamide (SK+F standard SJB-4406-226A) in water is shown in Figure 3. Maxima at 265 nm ($a = 0.745$; $\epsilon = 357.9$) and 258.5 nm ($a = 1.053$; $\epsilon = 505.9$) are bands characteristic for the mono-substituted phenyl moiety (1). Figure 4 is the ultraviolet spectrum in water under conditions suitable for observing the iodide maximum absorbance at 222.5 nm ($a = 37.0$; $\epsilon = 17,757$) (2, 3, 4). Beginning at 220 nm the automatic slit begins to rapidly open. Neither maximum wavelengths nor absorptivities different from those determined in water solution were observed in 0.1 N hydrochloric acid and 0.1 N sodium hydroxide solutions.

Figure 5 is the ultraviolet absorption spectrum of isopropamide in methylene chloride. The maximum at 245 nm ($a = 33.7$; $\epsilon = 16,204$) is due to the iodide. The automatic slit begins to rapidly open below 227.5 nm.

Figure 6 is the ultraviolet absorption curve of isopropamide in water after the solution was passed through an anionic exchange resin (5) in which the iodide was converted to the chloride; this allows the mono-substituted phenyl spectrum to be more readily observed. Maxima appear at 265 nm ($a = 0.721$; $\epsilon = 346.4$), 258.5 nm ($a = 0.940$; $\epsilon = 451.8$), and 252.5 nm ($a = 0.856$; $\epsilon = 411.5$). Neither maximum shifts nor absorptivity differences were observed in the above spectrum when the solution pH was rendered either acidic or basic.

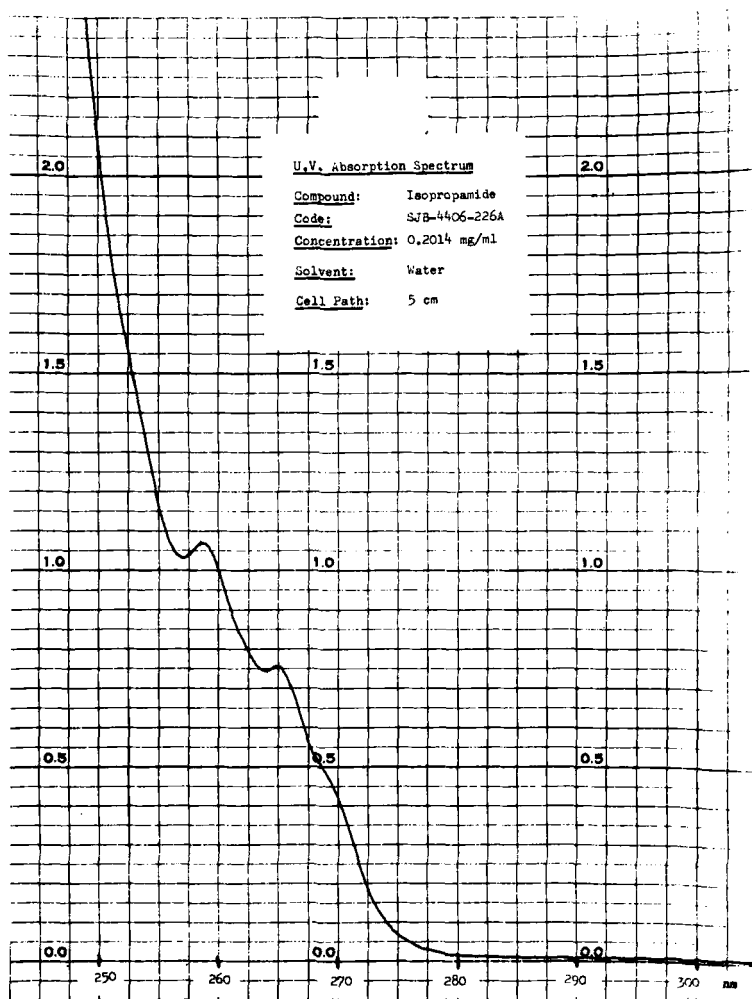


Fig. 3

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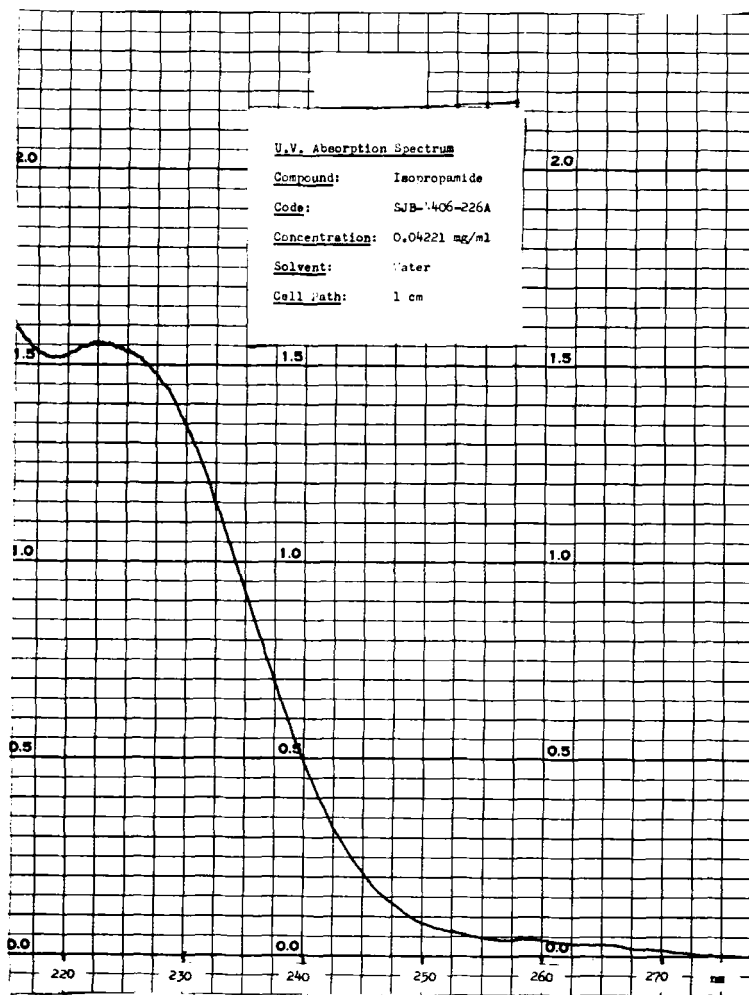


Fig. 4

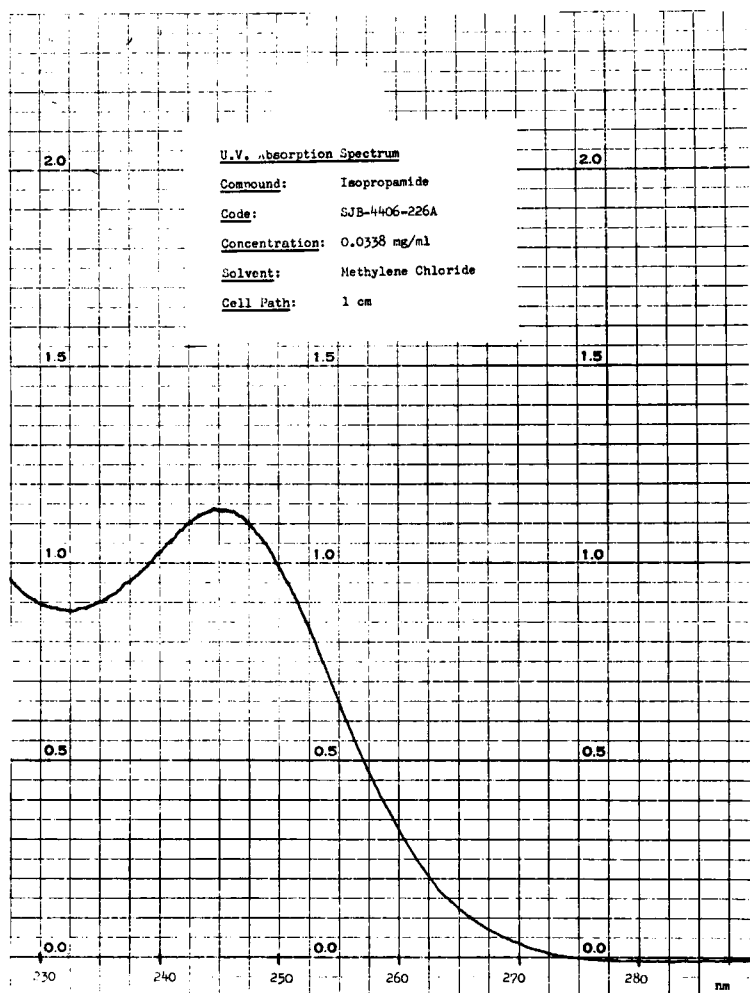


Fig. 5

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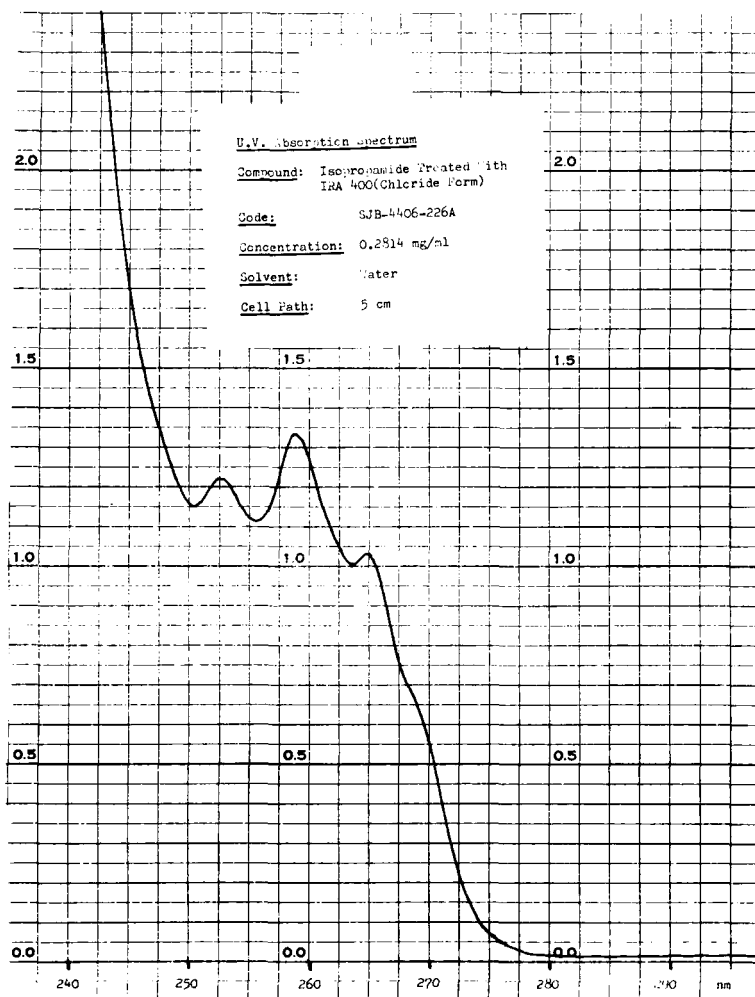


Fig. 6

2.4 Mass Spectrum

The mass spectrum of SK+F 4740-J (SK+F standard SJB-4406-226-A) was obtained by direct insertion of the solid into an Hitachi Perkin-Elmer RMU-6E low resolution mass spectrometer. The results are presented in tabular form in Table I and as a bar graph in Figure 7. E. White and G. Roberts provided the following observations and assignments:

The compound thermally decomposes in the instrument, and consequently no molecular ion is observed for the quaternary salt. The spectrum consists of the sum of the spectra of six thermal products which are listed in Table II. All of the structurally significant peaks are discussed below.

Compounds I, II, and III, tertiary amines show a molecular ion as noted in Table II. Each of the compounds cleaves β to the amino nitrogen by losing a methyl group resulting in peaks at m/e 100, m/e 295 and m/e 323 respectively. Compounds II and III also exhibit cleavage β to the amino nitrogen with strong peaks at m/e 86 and m/e 114 respectively. Following the loss of the methyl groups from the molecular ions of II and III there is loss of NH_2CHO resulting in peaks at m/e 250 and m/e 278. Following the loss of the methyl group from I there is loss of propene resulting in a peak at m/e 58 as transition is supported by a metastable at m/e 33.6

Compounds IV, V, and VI are alkyl iodides and as noted in Table 2 a molecular ion is observed for IV and V. Although no molecular ion is observed for VI, its presence is indicated by fragment ions. The peaks at m/e 238 and m/e 237 represent the loss of an iodine atom and hydrogen iodide. The ion at m/e 237 further decomposes to m/e 236 by loss of its hydrogen atom, this transition being supported by a metastable at m/e 235.0. The probable origins of m/e 193 and m/e 194 are the loss of NH_2CHO from m/e 238 and the loss of NHCO from m/e 237. The ions at m/e 178 and m/e 179 are possibly formed by loss of a methyl group from m/e 193 and m/e 194.

Other structurally significant peaks which could have originated from more than one of the six compounds

ISOPROPAMIDE

TABLE I

LOW RESOLUTION MASS SPECTRUM OF ISOPROPAMIDESK-F STANDARD SJB-4406-226A

MASS	INTEN.	MASS	INTEN.	MASS	INTEN.	MASS	INTEN.
15.0	0.5	79.0	1.9	137.0	0.4	202.0	0.5
16.0	0.2	80.0	0.5	138.0	0.4	203.0	0.5
19.0	0.5	81.0	0.9	139.0	4.1	204.0	0.9
20.0	0.5	82.0	4.2	140.0	1.3	205.0	0.4
26.0	0.2	83.0	3.7	141.0	1.9	206.0	2.2
27.0	23.5	84.0	5.6	142.0	12.5	207.0	0.9
28.0	5.6	85.0	2.7	143.0	0.3	208.0	1.6
29.0	3.5	86.0	77.5	146.0	0.4	209.0	1.4
30.0	11.7	87.0	5.3	149.0	0.5	210.0	2.1
31.0	0.9	88.0	2.2	150.0	1.1	211.0	6.8
32.0	0.4	89.0	9.2	151.0	2.2	212.0	0.8
33.0	0.8	90.0	1.8	152.0	4.5	218.0	0.4
34.0	1.9	91.0	14.9	153.0	2.3	219.0	1.1
36.0	0.9	92.0	1.6	154.0	0.8	220.0	0.6
37.0	2.0	93.0	0.4	155.0	0.7	221.0	0.3
38.0	3.4	94.0	1.5	156.0	0.5	222.0	0.9
39.0	20.4	95.0	1.4	159.0	0.5	223.0	0.3
40.0	7.0	96.0	2.1	160.0	2.3	225.0	0.4
41.0	49.7	97.0	0.4	161.0	0.6	234.0	0.4
42.0	24.5	98.0	1.7	162.0	0.4	235.0	3.2
43.0	58.8	99.0	2.0	163.0	4.7	236.0	86.0
44.0	29.6	100.0	89.2	164.0	5.5	237.0	35.1
45.0	15.0	101.0	7.6	165.0	49.7	238.0	36.4
46.0	0.4	102.0	3.7	166.0	13.2	239.0	6.5
50.0	1.3	103.0	13.9	167.0	7.6	240.0	0.7
51.0	6.5	104.0	4.7	168.0	1.4	250.0	6.0
52.0	1.4	105.0	1.5	170.0	26.3	251.0	2.3
53.0	0.6	106.0	0.6	171.0	0.9	252.0	0.7
54.0	1.5	107.0	0.4	172.0	0.3	253.0	0.3
55.0	2.1	109.0	0.4	174.0	0.5	264.0	1.4
56.0	21.4	110.0	0.4	175.0	0.4	265.0	0.3
57.0	6.9	111.0	0.4	176.0	2.6	267.0	3.3
58.0	100.0	112.0	0.5	177.0	4.1	268.0	0.8
59.0	4.3	113.0	1.4	178.0	33.8	276.0	1.2
60.0	0.5	114.0	31.5	179.0	31.9	277.0	0.3
62.0	1.6	115.0	48.8	180.0	11.7	278.0	4.0
63.0	3.7	116.0	25.1	181.0	2.3	279.0	1.7
64.0	0.7	117.0	17.3	182.0	5.1	280.0	0.4
65.0	3.4	118.0	3.7	183.0	0.8	292.0	0.2
67.0	0.5	119.0	0.4	185.0	0.3	295.0	5.4
68.0	1.1	120.0	0.5	187.0	0.4	296.0	1.2
69.0	0.9	121.0	0.3	188.0	0.6	297.0	0.2
70.0	6.3	125.0	0.4	189.0	3.0	305.0	0.3
71.0	5.9	126.0	1.5	190.0	4.4	309.0	0.4
72.0	16.8	127.0	18.9	191.0	7.5	310.0	0.8
73.0	1.5	128.0	18.1	192.0	28.6	311.0	0.2
74.0	0.9	129.0	1.2	193.0	61.6	323.0	2.8
75.0	2.1	130.0	1.0	194.0	33.3	324.0	0.4
76.0	4.8	131.0	0.4	195.0	4.2	338.0	0.8
77.0	18.8	132.0	1.4	196.0	0.4	339.0	0.3
78.0	2.8	133.0	0.4				

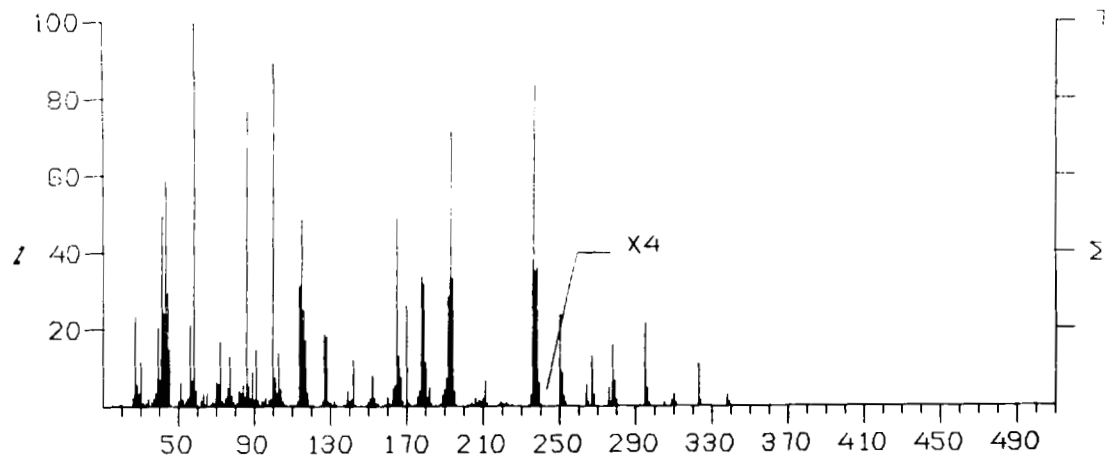


Figure 7. Low resolution mass spectrum of isopropamide, SK+F standard SJB-4406-226A.

are m/e 43 $[\text{CH}(\text{CH}_3)_2]^+$, m/e 127 (I^+), m/e 128 (HI^+) and m/e 165 $\left(\text{C}_{10}\text{H}_7^+\right)$,

TABLE II

Thermal Products of Isopropamide

I	$\text{CH}_3\text{N}[\text{CH}(\text{CH}_3)_2]_2$	$\text{M}^+ 115$
II	$\begin{array}{c} \text{O} \quad \emptyset \quad \text{CH}_3 \\ \parallel \quad \quad \\ \text{NH}_2 - \text{C} - \text{C} - \text{CH}_2\text{CH}_2\text{N} - \text{CH}(\text{CH}_3)_2 \\ \quad \quad \\ \quad \quad \emptyset \end{array}$	$\text{M}^+ 310$
III	$\begin{array}{c} \text{O} \quad \emptyset \\ \parallel \quad \\ \text{NH}_2 - \text{C} - \text{C} - \text{CH}_2\text{CH}_2\text{N}[\text{CH}(\text{CH}_3)_2]_2 \\ \quad \quad \\ \quad \quad \emptyset \end{array}$	$\text{M}^+ 338$
IV	CH_3I	$\text{M}^+ 142$
V	$(\text{CH}_3)_2\text{CHI}$	$\text{M}^+ 170$
VI	$\begin{array}{c} \text{O} \quad \emptyset \\ \parallel \quad \\ \text{NH}_2 - \text{C} - \text{C} - \text{CH}_2\text{CH}_2\text{I} \\ \quad \quad \\ \quad \quad \emptyset \end{array}$	M^+ not observed

2.5 Melting Range

Isopropamide (SK+F standard SJB-4406-226-A) melted between 189.0 - 191.5°C. without decomposition under USP conditions for class I substances (6).

2.6 Differential Thermal Analysis

The differential thermal analysis of isopropamide (SK+F standard SJB-4406-226A) beginning at 43°C and with a heating rate of 20°C/min. resulted in a single major melting endotherm at 200°C.

Other chemical lots tested under the same conditions showed minor endothermic transitions below the major endotherm. These minor transitions have been tentatively attributed to polymorphic forms known to exist with this compound (7).

2.7 Thermogravimetric Analysis

A thermogravimetric analysis performed on isopropamide (SK+F standard SJB-4406-226-A) showed a 0.5% loss in weight complete at about 105°C. The measurement was performed under nitrogen sweep at a heating rate of 10°C/min. Additional weight was rapidly lost as the sample began melting at approximately 185°C.

2.8 Solubility

The following solubilities were obtained at room temperature:

<u>Solvent</u>	<u>Solution</u>	<u>conc.</u> <u>mg/ml</u>
ether	colorless	0.07
benzene	colorless	0.09
cyclohexane	colorless	0.10
pH 7.5 buffer	colorless	20.7
water	colorless	21.2
0.1 N hydrochloric acid	faint yellow	23.6
0.1 N sodium hydroxide	colorless	23.6
acetone	bright yellow	24.9
95% ethanol	faint yellow	109.0
methylene chloride	brownish yellow	330.4
chloroform	deep brownish yellow	> 500
methanol	deep brownish yellow	> 500

In addition, the following solubilities at room temperature have been reported (8):

<u>Solvent</u>	<u>Conc.</u> <u>mg/ml</u>
Ethyl acetate	0.1
1,1, dichloroethane	1.3
isopropanol	3.2
Methylethylketone	4.0
1 N hydrochloric acid	29.0

2.9 Polarography

The polarogram was obtained on isopropamide (SK+F standard SJB-4406-226A) in 0.1 N potassium nitrate with a standard calomel/dropping mercury electrode system at a concentration of 5×10^{-4} molar (9). The $E_{1/2}$ was -0.254 V. The $E_{1/2}$ for iodide determined under similar conditions was -0.225 V.

3. Synthesis

The synthetic pathway to isopropamide includes the following steps (Figure 8):

2-Diisopropylaminoethyl chloride (I) is condensed with diphenylacetoneitrile (II) in the presence of an alkaline condensing agent and the product 4-diisopropylamino-2,2-diphenylbutyronitrile (III) purified by distillation.

By reaction with acid, the 4-diisopropylamino-2,2-diphenylbutyronitrile (III) is partially hydrolyzed to 4-diisopropylamino-2,2-diphenylbutyramide (IV) which is purified by recrystallization.

Finally (3-carbamoyl-3,3-diphenylpropyl)-diisopropyl-methylammonium iodide (V) is prepared by reacting 4-diisopropylamino-2,2-diphenylbutyramide (IV) in solution with methyl iodide. The product is collected by filtration, washed, dried and assayed.

Isopropamide is covered by U.S. Patent Number 2823233. The original synthesis is described by Janssen *et al.* (10).

4 Stability - Degradation

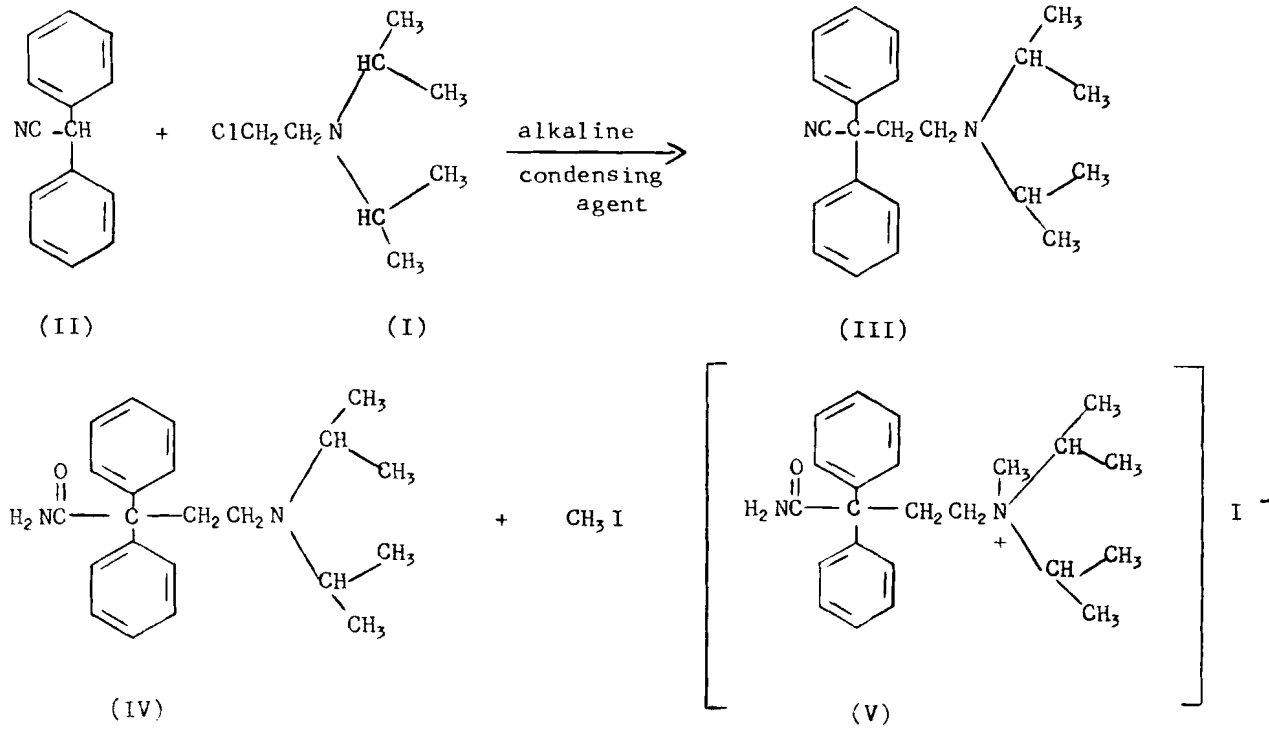
The dry chemical stored in glass bottles at room temperature is stable for at least 2 years. In aqueous solution (1 - 5 mg/ml) stored at room temperature the drug is stable for at least 6 months (11). Because the drug is stable, degradation products have not been observed under to occur either under normal or exaggerated storage conditions.

5. Drug Metabolic Products

No isopropamide metabolic products have been reported thus far.

Figure 8

SYNTHETIC PATHWAY TO ISOPROPAMIDE



6. Methods of Analysis

6.1 Isopropamide Chemical

6.11 Elemental Analysis

<u>Element</u>	<u>% Theory</u>	<u>Range Obtained</u>
C	57.50	57.80 - 57.92
H	6.90	7.00 - 7.06
N	5.83	5.69 - 5.78

6.12 Titration of Iodide Functional Group

An accurately weighed sample (About 1 g.) is dissolved in a 5:5:1 mixture of water:methyl alcohol:glacial acetic acid and titrated with 0.1 N silver nitrate and Eosin Y as indicator until the precipitate becomes rose red. Each milliliter of 0.1 N silver nitrate is equal to 0.01269 of iodide or 0.04804 g. of isopropamide.

6.13 Non Aqueous Titration

An accurately weighed sample (about 0.75 g.) is dissolved in glacial acetic acid, mercuric acetate and methyl violet indicator added, and titrated with 0.1 N perchloric acid in acetic acid to a blue endpoint. Each milliliter of 0.1 N perchloric acid is equivalent to 0.04804 g. of isopropamide.

6.14 Spectrophotometric Analysis

The ultraviolet absorption spectrum obtained either by direct dilution or preliminary treatment with resin (see section 2.3) may be used for determining purity.

6.15 Chromatographic Analysis

The following thin layer method may be used for the qualitative purity evaluation of isopropamide:

Equilibrate a mixture of 60 ml. each of isoamyl and tert-amyl alcohols with 20 ml. of 88% formic acid dissolved in 100 ml. of water. Discard the aqueous layer and use the alcohol layer as the chromatographic solvent. Spot 25 and 50 micrograms of isopropamide dissolved in methanol two cm. from the edge of an 'Avicel'

or cellulose plate (12), place the prepared plate in a suitable chromatographic chamber lined with filter paper saturated with the developing solvent, and allow to equilibrate for 45 minutes. Allow the solvent to rise to a line drawn across the plate 10 cm. from the origin, remove the plate, and air dry in a fume hood until solvent vapors are no longer detectable. The developed chromatogram may be visibilized under ultraviolet light (254 and 365 nm), visible light, and iodoplatinate reagent (13). The quaternary moiety has an approximate R_f of 0.8, while the iodide appears at approximately 0.2.

6.2 Dosage Forms

Isopropamide is found in dosage forms both alone and in combination with primary and tertiary amines. Assay methods include ultraviolet analysis after extraction of interfering amines and treatment with an anionic exchange resin, and colorimetric analysis by ion-pairing with acid dyes and extraction of the complex into an organic solvent.

6.21 Spectrophotometric Analysis

The ultraviolet assay of tablets which contain only isopropamide as the active ingredient is the most direct method employed (14). Twenty tablets (5 mg/tablet) are placed in a 250 ml. volumetric flask, disintegrated in about 150 ml. of water, and diluted to volume with water. The solution is filtered through Whatman No. 1 filter paper (15), 50.0 ml. of the filtrate percolated through an anion exchange column (16), and the column rinsed thoroughly with water. All eluates are collected in a 100 ml. volumetric flask and diluted to volume with water. The ultraviolet absorption spectrum of this solution is compared with that of a known standard of isopropamide under the same spectrophotometric conditions and the assay value per tablet calculated.

6.22 Spectrophotometric Analysis in Presence of Interferences

The ultraviolet assay of dosage forms which contain other amines removable by solvent extraction is accomplished by treating the eluate collected in 6.21 with ammonium hydroxide until basic and extracting with ether to remove the interfering amines (17).

6.23 Colorimetric Analysis

The selective determination of isopropamide by ion-pairing with methyl orange dye has been described (18). The quaternary amine is ion-paired at pH 10.2 and extracted into chloroform. Primary, secondary, and tertiary amines tested through the procedure did not complex or interfere with the subsequent spectrophotometric determination at 520 nm.

7. Notes and References

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8. Paul Janssen, Private Communication, Eupharma-Ned-Chem Pharmaceutical Laboratories.
9. Kolthoff, I.M., and Lingane, J.J., Polarography, Second Edition, Interscience Publishers, New York and London, 1952, p. 579.
10. Janssen et al., Arch. Intern Pharmacodyn., 103, 1955, 82.
11. Private Communication, Eupharma-Nedchem Pharmaceutical Laboratories.
12. 'Avicel'-microcrystalline cellulose - American Viscose. Silica gel may cause decomposition of the compound and is to be avoided.
13. One gram of chloroplatinic acid, 10 ml. of 1 N hydrochloric acid, and 5 g. of potassium iodide diluted to 250 ml. with water as a stock solution. Refrigerate. Prepare a working solution with equal volumes of stock solution and water made 1/2% with 88% formic acid.
14. M. Kushner, Personal Communication.
15. If the solution is not clear at this point it will be necessary to add 5 ml. of 10% aluminum chloride and 2 ml. of concentrated ammonium hydroxide to the 150 ml. of water before diluting to volume.
16. Amberlite IRA 400, chloride form.
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18. R. Santoro, J. Am. Pharm. Assoc., Sci. Ed., 49, 1960, 666.

Acknowledgements

The authors wish to thank Joan Rudin for her search of the literature and Patricia Brittingham for her invaluable secretarial help.

LEVALLORPHAN TARTRATE

Bruce C. Rudy and Bernard Z. Senkowski

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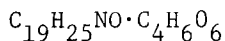
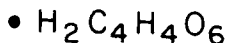
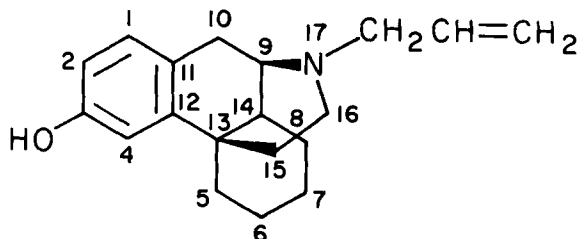
1. Description
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LEVALLORPHAN TARTRATE

1. Description

1.1 Name, Formula, Molecular Weight

Levallorphan tartrate is (-)-17-allylmorphinan-3-ol tartrate (1:1).



Molecular Weight: 433.51

1.2 Appearance, Color, Odor

Levallorphan tartrate occurs as a white or practically white, odorless, crystalline powder.

2. Physical Properties

2.1 Infrared Spectrum (IR)

The IR spectrum of levallorphan tartrate is greatly complicated by the presence of the tartaric acid. To obtain the IR spectrum characteristic of the active species, the free levallorphan base was extracted from a basic solution, dried, and measured as a KBr dispersion (1.7 mg levallorphan/300 mg KBr) on a Perkin Elmer 621 Spectrophotometer. This spectrum is shown in Figure 1 and the assignments for the characteristic bands are given in Table I (1).

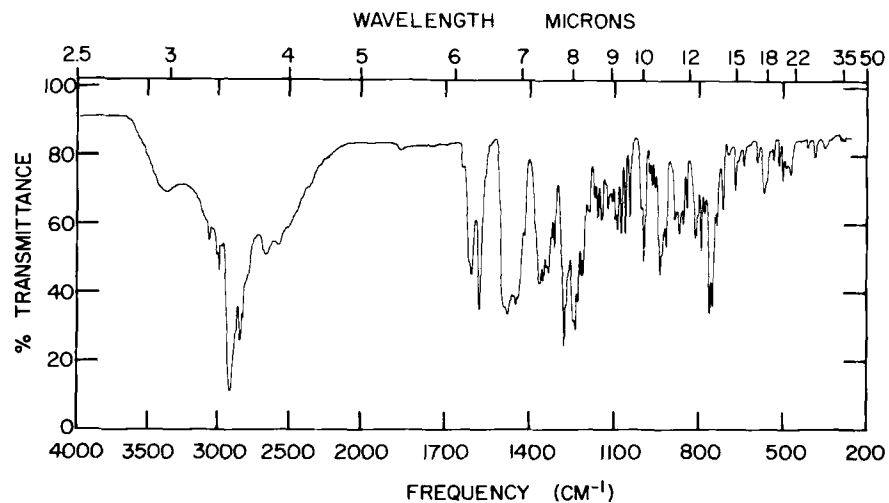
Table I

Infrared Assignments for Levallorphan

<u>Frequency (cm⁻¹)</u>	<u>Characteristic of</u>
3580*	OH stretch
3076	Aromatic CH stretch
2924-2919 and 2851	Asymmetric and symmetric stretch of CH ₂
1644	C=C stretch of vinyl group

*Detectable only in solution spectrum, not in KBr pellet.

Figure 1
Infrared Spectrum of Levallorphan



1607	Aromatic skeletal vibrations
1454	CH ₂ deformations
992 and 910	CH ₂ out of plane bending of vinyl group

2.2 Nuclear Magnetic Resonance Spectrum (NMR)

The spectra of levallorphan tartrate (A) and levallorphan base (B) are shown in Figure 2. These spectra were obtained by dissolving about 63 mg of the appropriate sample in 0.5 ml of DMSO-d₆ containing tetramethylsilane as an internal standard (2). The spectral assignments for the levallorphan base are given in Table II. Aside from the additional peaks from the protons on the tartrate itself,* the major difference between the base and the tartrate salt occurs in the methylene region. The broad, non-descript bands are attributed to the change in the nitrogen quadropole moment caused by the positive charge present on the nitrogen.

Table II

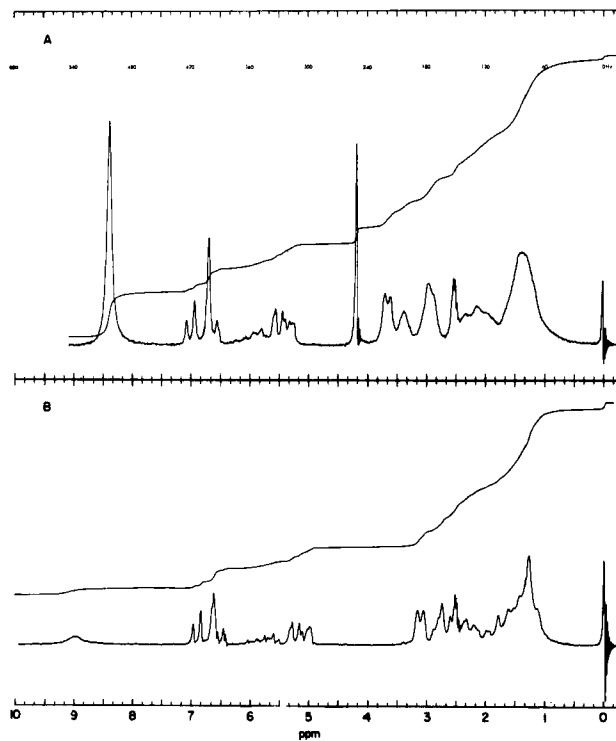
NMR Assignments for Levallorphan Base

<u>Type of Protons</u>	<u>No. of Protons</u>	<u>Chemical Shift (ppm)</u>	<u>Multiplicity</u>
methylene protons on carbons 5,6,7, 8, and 15	10	1.00-1.95	
methylene protons on carbon 10 and methyne proton on carbon 14	3	2.10-2.65	
methylene protons on carbon 16 and methyne proton on carbon 9	3	2.75-3.15	

*The tartrate has 2 C-H protons which show a sharp singlet at 4.10 ppm and 2 OH plus 2 COOH protons which along with the OH proton from the levallorphan molecule give a broad singlet at 8.49 ppm.

Figure 2

NMR Spectra of Levallorphan and Levallorphan Tartrate



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methylene protons adjacent to the N in allyl chain	2	3.10	Doublet (J=7Hz)
methylene protons at end of allyl group	2	4.95-5.25	Multiplet
methyne proton in allyl group	1	5.50-6.10	Multiplet
aromatic protons at carbons 2 and 4	2	6.40-6.61	Multiplet
aromatic proton at carbon 1	1	6.90	Doublet (J=8Hz)
OH proton	1	9.00	Broad singlet

2.3 Ultraviolet Spectrum (UV)

The UV spectra of levallorphan tartrate in water (A) and in 1N NaOH (B) are shown in Figure 3 (3). The maxima and minima as well as the molar extinction coefficients at the λ_{max} are listed in Table III. The values obtained agree well with the ones reported by Farmilo and Genest (4) and Mulé (5).

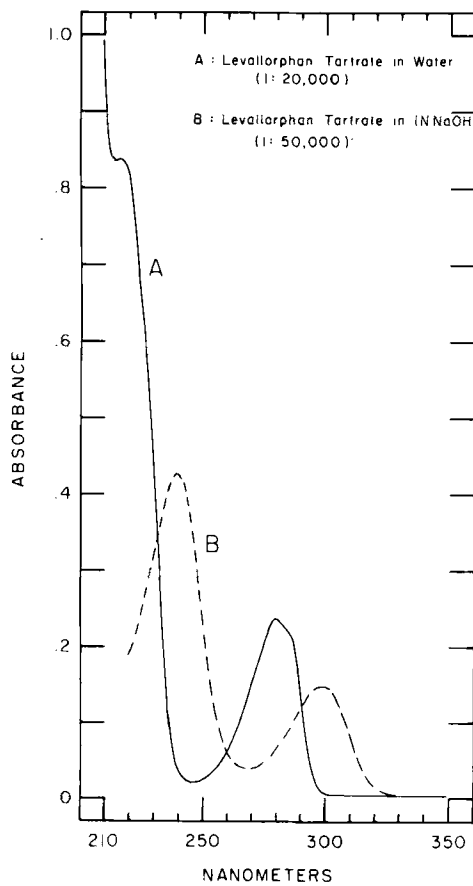
Table III

UV Absorption Spectral Data for Levallorphan Tartrate

<u>Solvent</u>	<u>Wavelength of Maximum (nm)</u>	<u>Molar Absorptivity</u>	<u>Wavelength of Minimum (nm)</u>
water	279	2.04×10^3	245
1N HCl	216	7.25×10^3	
	278	2.08×10^3	244
1N NaOH	216	7.42×10^3	
	299	3.18×10^3	268
	239	9.18×10^3	

Figure 3

Ultraviolet Spectrum of Levallorphan Tartrate



2.4 Fluorescence Spectrum

The excitation and emission spectra for levallorphan tartrate (1 mg/ml of methanol) is shown in Figure 4 (6). Two maxima appear in the excitation spectrum at 306 nm. When 0.1N HCl is used as the solvent the maxima occur at the same wavelengths but a 5 fold increase in sensitivity is obtained.

2.5 Mass Spectrum

The mass spectrum of levallorphan tartrate was obtained using a CEC 21-110 mass spectrometer with an ionizing energy of 70 eV. The output from the mass spectrometer was analyzed and presented in the form of a bar graph, shown in Figure 5, by a Varian 100 MS dedicated computer system (7). The mass spectrum is a superimposition of the spectra of the levallorphan base and tartaric acid. The molecular ion of levallorphan at m/e 283 is the strongest peak. The peak at m/e 256 (M-27) corresponds to the loss of $\text{CH}=\text{CH}_2$. The other peaks at m/e 268, 242, 240, and 226 are due to the loss of other hydrocarbon moieties. Since the polycyclic saturated ring system allows a multitude of fragmentation pathways, an assignment of these ions to certain parts of the molecular structure could at best be very tentative (7).

2.6 Optical Rotation

The specific rotation of levallorphan tartrate in water at 25°C is plotted versus wavelength in Figure 6 (3, 8). The specific rotation observed at 589 nm was -39.0° . The characteristic parts of the curve are a peak at 290 nm (-1165°), a trough at 267 nm ($+240^\circ$) and a peak at 220 nm (-5854°). Zero intercepts occur at 270 and 264 nm (8).

The specific rotation range reported in the USP XVIII for levallorphan tartrate is from -37.0 to -39.2° at 589 nm (9).

2.7 Melting Range

The melting range reported in the USP XVIII for levallorphan tartrate is 174° to 177° using the class Ia procedure (9).

2.8 Differential Scanning Calorimetry (DSC)

The DSC curve shown in Figure 7 for levallorphan tartrate was obtained using a Perkin Elmer DSC-1B Calorimeter. With a temperature program of $10^\circ\text{C}/\text{min}$, a melting

Figure 4

Fluorescence Spectrum of Levallorphan Tartrate

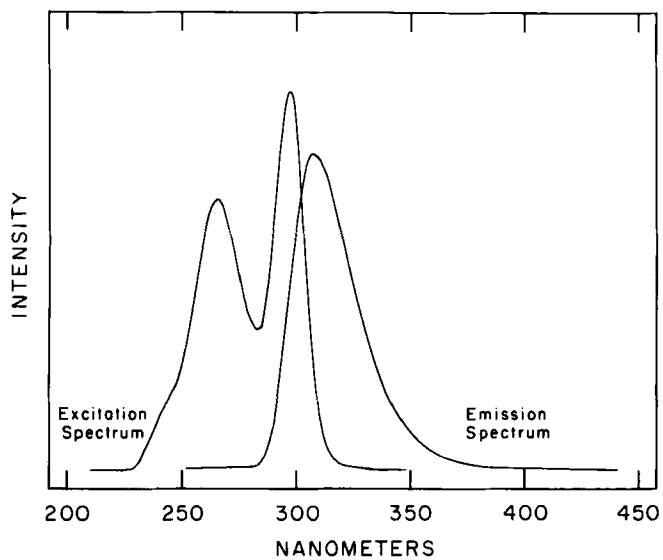


Figure 5

Mass Spectrum of Levallorphan Tartrate

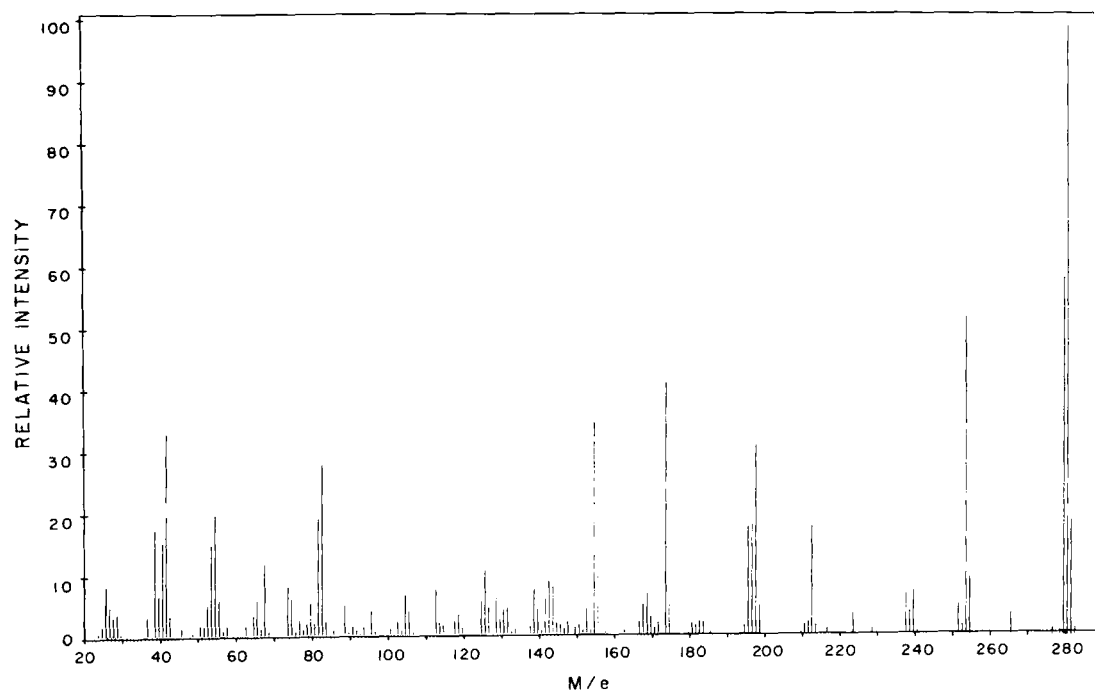
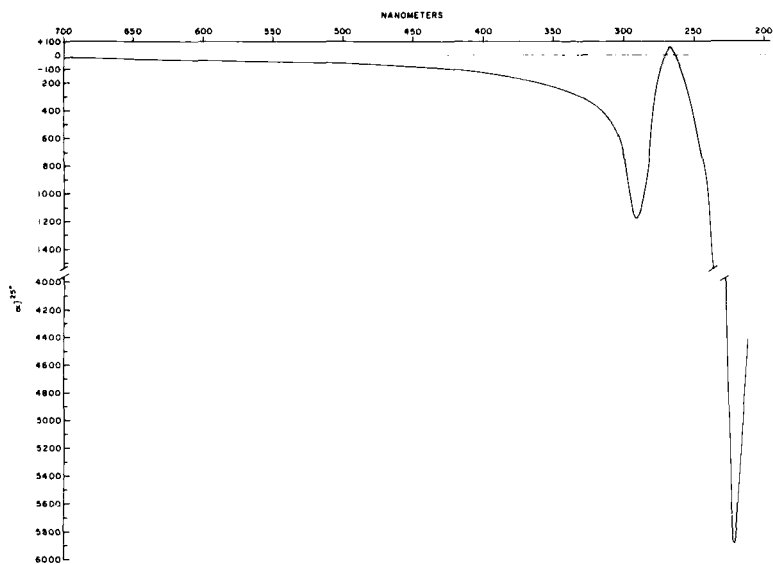


Figure 6

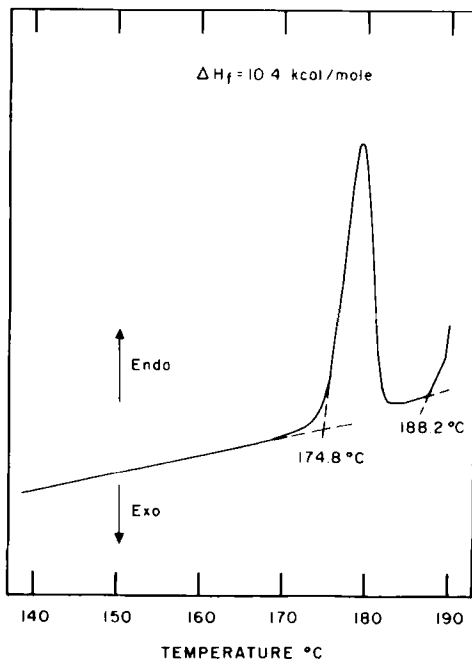
Plot of Specific Rotation vs. Wavelength
of Levallorphan Tartrate



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Figure 7

DSC Curve of Levallorphan Tartrate



endotherm was observed starting at 174.8° with a $\Delta H_f = 10.4$ kcal/mole (10).

2.9 Thermogravimetric Analysis (TGA)

The TGA scan run on levallorphan tartrate showed no weight loss from 30° to 217°C. One continuous weight loss occurred from 217° to 470°C which accounted for essentially all of the sample (10).

2.10 Solubility

The solubility data obtained for levallorphan tartrate at 25°C are listed in Table IV (11).

Table IV

Solubility of Levallorphan Tartrate in Different Solvents

<u>Solvent</u>	<u>Solubility (mg/ml)</u>
3A alcohol	7.8
benzene	0.2
chloroform	0.3
95% ethanol	26.5
ethyl ether	0.2
methanol	79.5
petroleum ether (30°-60°)	<0.03
2-propanol	1.5
water	60.0

2.11 X-ray Crystal Properties

The x-ray powder diffraction pattern of levallorphan tartrate is presented in Table V (12). These values are very similar to those given by Farmilo and Genest (4). The instrumental conditions are given below.

Instrument Conditions:

General Electric Model XRD-6 Spectrogoniometer

Generator:	50KV, 12-1/2 MA
Tube target:	Copper
Radiation:	Cu $K_\alpha = 1.542 \text{ \AA}$

LEVALLORPHAN TARTRATE

Optics: 0.1° Detector slit
M.R. Soller slit
3° Beam slit
0.0007 inch Ni filter
4° take off angle
Goniometer: Scan at 0.2° 2θ per minute
Detector: Amplifier gain - 16 course,
8.7 fine
Sealed proportional counter
tube and DC voltage at
plateau
Pulse height selection E_L-5
volts; Eu - out
Rate meter T.C. 4
2000 C/S full scale
Recorder: Chart Speed - 1 inch per 5
minutes

Samples prepared by grinding at room temperature.

Table V

X-ray Powder Diffraction Pattern of Levallorphan Tartrate

<u>2θ</u>	<u>d(Å)*</u>	<u>I/I₀**</u>	<u>2θ</u>	<u>d(Å)*</u>	<u>I/I₀**</u>
9.18	9.63	30	29.18	3.06	23
11.90	7.44	33	29.66	3.01	10
12.18	7.27	15	30.10	2.97	1
13.06	6.78	78	30.24	2.96	2
14.12	6.27	10	30.38	2.94	2
14.28	6.20	32	30.92	2.89	10
15.32	5.78	100	31.08	2.88	11
15.84	5.59	32	31.74	2.82	14
17.22	5.15	6	31.86	2.81	17
18.50	4.80	21	34.14	2.63	1
19.00	4.67	12	34.30	2.61	1
19.16	4.63	34	35.02	2.56	11
19.70	4.51	10	35.16	2.55	13
19.90	4.46	34	35.32	2.54	12
20.18	4.40	32	35.70	2.51	5
20.78	4.27	22	35.86	2.50	5
21.00	4.23	49	36.22	2.48	2
21.48	4.14	14	36.72	2.45	5

21.76	4.08	39	37.38	2.41	1
23.12	3.85	6	38.08	2.36	4
23.88	3.73	11	38.40	2.34	6
24.06	3.70	11	38.80	2.32	33
24.62	3.62	32	39.40	2.29	1
24.86	3.58	85	39.96	2.26	4
25.58	3.48	5	40.06	2.25	6
26.00	3.43	18	40.24	2.24	7
26.16	3.41	18	40.52	2.23	4
26.42	3.37	34	40.92	2.21	7
26.78	3.33	1	41.74	2.16	1
27.22	3.28	9	42.04	2.15	6
27.58	3.24	8	43.62	2.07	3
27.98	3.19	4	43.82	2.07	5
28.74	3.11	11	44.08	2.05	13
28.84	3.10	16	45.14	2.01	6

$$* d - (\text{interplanar distance}) \frac{n\lambda}{2 \sin \theta}$$

$$** I/I_0 = \text{relative intensity (based on highest intensity of 1.00)}$$

2.12 Dissociation Constant

The pK_a 's at room temperature for levallorphan tartrate have been determined by a pH titration with sodium hydroxide to be 4.5 and 6.9 (13). The first pK_a observed for levallorphan tartrate results from the ionization of the carboxylic proton from the tartaric acid. The second pK_a is due to the ionization of the proton from the ammonium moiety in the levallorphan tartrate.

3. Synthesis

Levallorphan tartrate may be prepared by the reaction scheme shown in Figure 8. (+)-1-(p-hydroxybenzyl)-1,2,3,4,5,6,7,8-octahydroisoquinoline is reacted with allylbromide to give (-)-1-(p-hydroxybenzyl)-2-allyl-1,2,3,4,5,6,7,8-octahydroisoquinoline which is cyclized in phosphoric acid to (-)-3-hydroxy-17-allyl-morphinan. Tartaric acid is then added to make levallorphan tartrate (14).

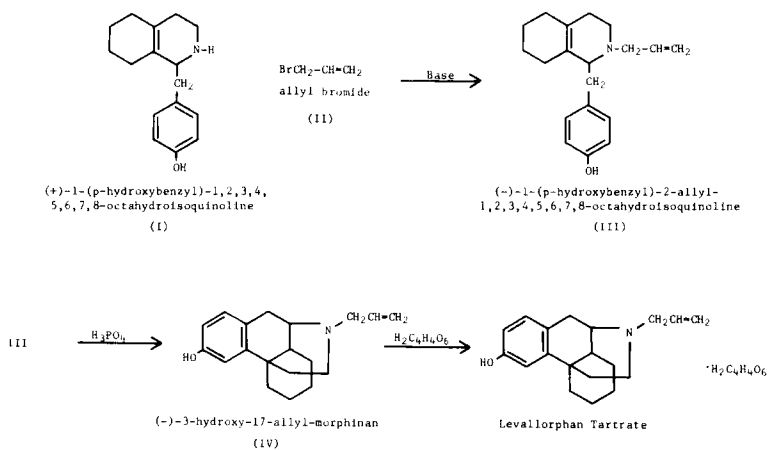
4. Stability Degradation

Levallorphan tartrate substance is stable in the presence of light and oxygen from the air. In aqueous

LEVALLORPHAN TARTRATE

Figure 8

SYNTHESIS OF LEVALLORPHAN TARTRATE



solution levallorphan tartrate was shown to be stable when heated to 45°C for 12 weeks. Only when the solution was saturated with oxygen and subjected to ultra violet radiation did slow decomposition occur. The levallorphan tartrate shows no effects from sterilization at 120°C for 20 minutes (15).

5. Drug Metabolic Products

The metabolic fate of levallorphan tartrate in animals has been studied by Mannering and Schanker (16). Two metabolic products were isolated from urine, feces, and the liver of mice. One of the metabolites was positively identified as the N-dealkylated product, 3-hydroxymorphinan. The other metabolite was not identified until recently when it was shown to be (-)-17-allyl-3,6-dehydroxymorphinan by x-ray crystallography (17). The 3-hydroxymorphinan metabolite was also found in the urine of guinea pigs, rabbits, and dogs (16).

6. Methods of Analysis

6.1 Elemental Analysis

The results from the elemental analysis of levallorphan tartrate are listed in Table VI (18).

Table VI

Elemental Analysis of Levallorphan Tartrate

<u>Element</u>	<u>% Theory</u>	<u>% Found</u>
C	63.73	63.77
H	7.21	7.26
N	3.23	3.22

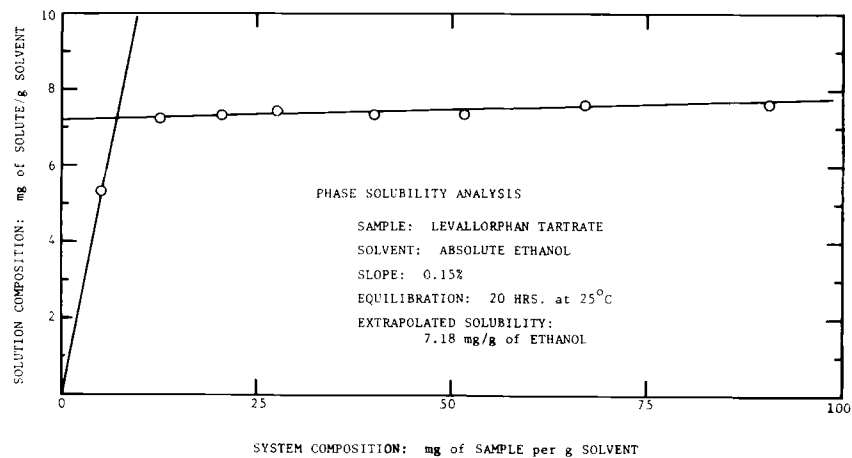
6.2 Phase Solubility Analysis

Phase solubility analysis is carried out using absolute ethanol as the solvent. A typical example is shown in Figure 9 which also lists the conditions under which the analysis was carried out (11).

6.3 Thin Layer Chromatographic Analysis (TLC)

Several TLC systems have been published which can be used to separate levallorphan base from its metabolites

Figure 9



and other similar compounds (5,19). In each case about 25 μ g of sample was spotted on a Silica gel G plate (250 microns thick) and subjected to ascending chromatography. After development of at least 10 cm, the plate is air-dried for 10 minutes, dried in an oven at 100°C for 15 minutes, and sprayed with iodoplatinate reagent made by adding 10 ml of a 10% solution of platinum chloride to 250 ml of 4% potassium iodide and diluting to 500 ml with water. The solvent systems as well as the approximate R_f of the levallorphan spot are listed in Table VII (5).

Table VII

TLC Data for Levallorphan

<u>Solvent System</u>	<u>R_f of Levallorphan</u>
benzene:dioxane:ethanol:ammonium hydroxide (10:8:1:1)	.98
n-butanol:conc. hydrochloric acid, saturated with water (9:1)	.73
ethanol:glacial acetic acid:water (6:3:1)	.70
ethanol:dioxane:pyridine:water (10:5:4:1)	.65
n-butanol:water:glacial acetic acid (4:2:1)	.64
t-amyl alcohol:water:n-butyl ether (80:13:7)	.44
methanol:n-butanol:water:benzene (12:3:3:2)	.41

6.4 Gas Liquid Chromatographic Analysis (GLC)

A GLC method for the separation and quantitation of levallorphan base in the presence of its metabolites and similar structured compounds has been reported by Mulé (5, 19). The pertinent experimental conditions as well as the retention time for levallorphan are given in Table VIII (5).

Table VIII

GLC Method for Levallorphan as the Free Base

Column:	6 ft, 3 mm id, coiled glass
Support:	Gas Chrom S (80-100 mesh)
Stationary Phase:	2% SE-30
Detector:	Radium - 226 argon ionization
Temperature -	
Injection Port:	260°
Column:	215°
Detector:	215°
Flow Rate:	47 cc/minute of argon
Quantities Injected:	2-25 µg of levallorphan base in methanol
Retention Time:	5.72 minutes

6.5 Direct Spectrophotometric Analysis

Direct spectrophotometric analysis of levallorphan tartrate has not been found to be applicable if any metabolites or similar structured compounds are present. However, if no interfering species are present, the reported maximum at 279 nm in water and in 1N HCl or at 299 nm in 1N NaOH may be used for quantitative measurements.

6.6 Colorimetric Analysis6.61 Ion Pair Extraction of Bromocresol Green Complex

A colorimetric method which takes advantage of the quaternary amine moiety in levallorphan tartrate is the extraction of the ion pair formed with bromocresol green. The levallorphan tartrate in injectables is assayed by this method (9). The complex is formed in an aqueous 5.3 phosphate buffer and extracted into chloroform. The absorbance is read at the 420 nm maximum along with the absorbance of a reference standard levallorphan tartrate prepared in the same way. These absorbance values are used to calculate the concentration of levallorphan tartrate per ml present in the injectable.

6.62 Folin-Ciocalteu Colorimetric Analysis

A colorimetric method which takes advantage of the phenol moiety in levallorphan tartrate utilizes the

Folin-Ciocalteu reagent (20). This reagent is prepared by mixing 100 gm of sodium tungstate, 25 gm of sodium molybdate, 700 ml of water, 50 ml of 85% phosphoric acid, and 100 ml of concentrated hydrochloric acid. After refluxing for 10 hours, 150 gm of lithium sulfate, 50 ml of water, and a few drops of liquid bromine are added and the resulting solution boiled a few minutes to drive off excess bromine. This solution is cooled, diluted to 1000 ml and filtered (20). When this reagent is added to levallorphan tartrate in a slightly basic solution (sodium carbonate) a blue color develops which is measured at the maximum at about 700 nm.

6.7 Nephelometric Analysis

A nephelometric method was developed for the analysis of levallorphan tartrate in the presence of levorphanol tartrate. The method takes advantage of the kinetics of bromination of the allyl function on levallorphan which in acidic aqueous medium is faster than the bromination of the levorphanol. The bromination product is insoluble and hence lends itself to a turbidimetric determination (21). The procedure involves adding 1 mg of levallorphan tartrate (along with up to 10 mg of levorphanol tartrate) to 5 ml of 2.5N HCl and 15 ml of water in a glass stoppered 25-ml graduated cylinder. Bring to 25°C and add 3.0 ml of 0.1N bromide-bromate solution, shake and maintain at 25°C for exactly 45 minutes. Shake and measure the absorbance at 640 nm. A plot of absorbance vs concentration is linear between 0.5 and 1.5 mg of levallorphan tartrate (21).

6.8 Titrimetric Analysis

The potentiometric, non aqueous titration is the method of choice for analyzing levallorphan tartrate fine chemical (9). About 250 mg of levallorphan tartrate is dissolved in 100 ml of glacial acetic acid and titrated with 0.02N perchloric acid in dioxane. Each ml of 0.02N perchloric acid is equivalent to 8.67 mg of levallorphan tartrate.

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METHYPRYLON
An Analytical Profile

Bruce C. Rudy and Bernard Z. Senkowski

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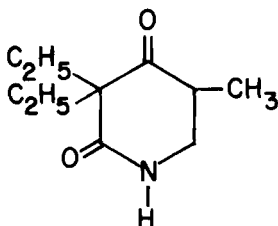
Analytical Profile - Methypylon

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 - 1.2 Appearance, Color, Odor
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1. Description

1.1 Name, Formula, Molecular Weight

Methyprylon is 3,3-diethyl-5-methyl-2,4-piperidinedione.



$C_{10}H_{17}NO_2$

Molecular Weight: 183.25

1.2 Appearance, Color, Odor

Methyprylon occurs as a white to nearly white crystalline powder. It has a slight characteristic odor.

2. Physical Properties

2.1 Infrared Spectrum (IR)

The IR spectrum of methyprylon is presented in Figure 1 (1). The spectrum was measured with a Perkin-Elmer 621 Spectrophotometer in a KBr pellet containing 0.5 mg of methyprylon/300 mg of KBr. The assignments for the characteristic bands in the IR spectrum are listed below (1):

3345 cm^{-1}	NH stretching vibrations
2966, 2933, and 2877 cm^{-1}	aliphatic CH stretching vibrations
1693 and 1656 cm^{-1}	C=O stretching vibrations

2.2 Nuclear Magnetic Resonance Spectrum (NMR)

The spectrum shown in Figure 2 was obtained on a Jeol 60 MHz NMR by dissolving 59.0 mg of methyprylon in 0.5 ml of $CDCl_3$ containing tetramethylsilane as an internal reference (2). The spectral assignments are given in Table I (2). In order to make these assignments and establish the chemical shifts, extensive spin-spin decoupling experiments were carried out. By irradiating the sample at the

Figure 1
Infrared Spectrum of Methypylon

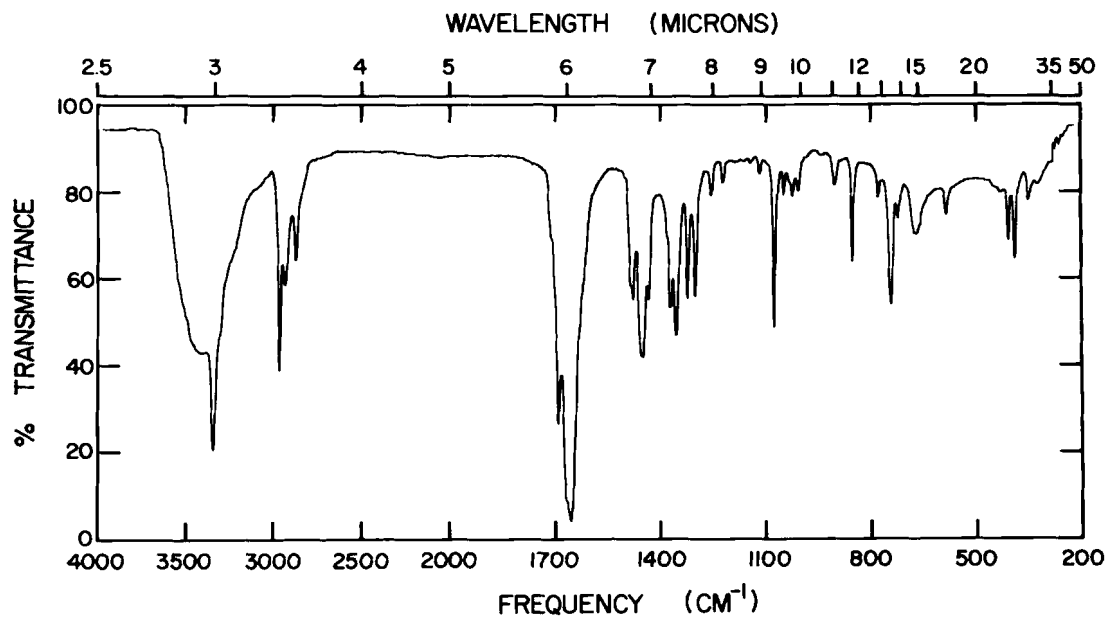
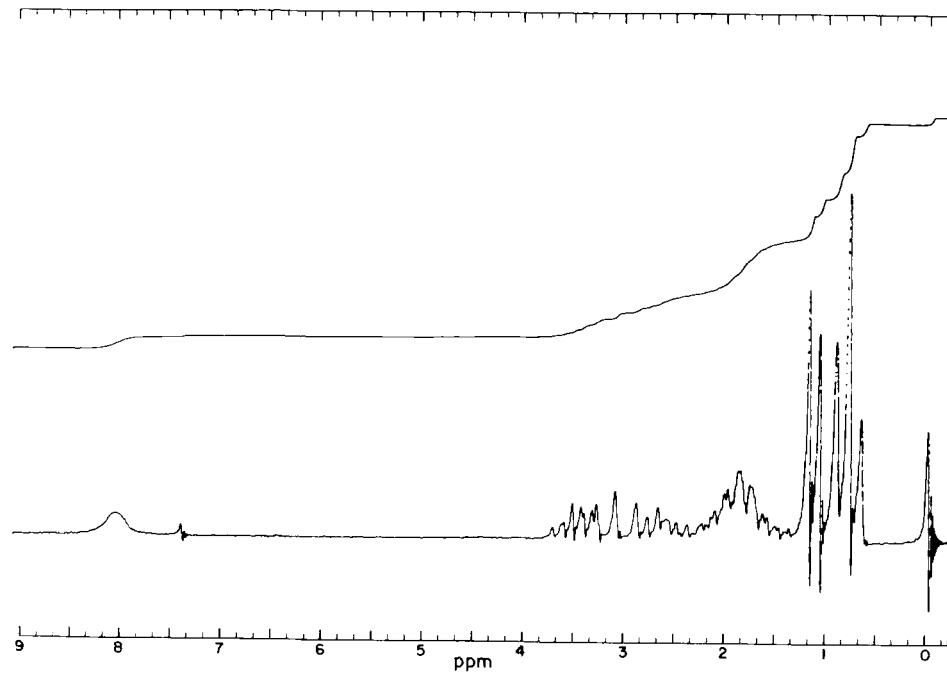


Figure 2

NMR Spectrum of Methyprylon

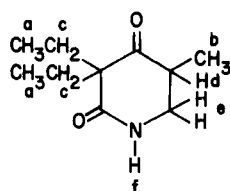


METHYPRYLON

frequencies 48, 65, 114, and 206 Hz, the spectrum was simplified and assignments could then be made (2).

Table I

NMR Assignments for Methyprylon

<u>Structure</u>	<u>Protons</u>	<u>Chemical Shift (ppm)</u>	<u>Multiplicity</u>
	a	0.8	Triplet ($J_{H_a-H_e} = 7.5 \text{ Hz}$)
	b	1.14	Doublet ($J_{H_b-H_d} = 7.0 \text{ Hz}$)
	c	~ 1.90	Complex Multiplet ($J_{H_e-H_a} = 7.5 \text{ Hz}$)
	d	~ 2.50	Complex Multiplet ($J_{H_d-H_b} = 6.8 \text{ Hz}$) ($J_{H_d-H_e} = 4.0 \text{ Hz}$)
	e	3.41	Nonet ($J_{H_e-H_f} = 7.0 \text{ Hz}$) ($J_{H_e-H_d} = 6.0 \text{ Hz}$) ($J_{H_e-H_b} = \text{Unknown}$)
	f	8.00	Broad Singlet

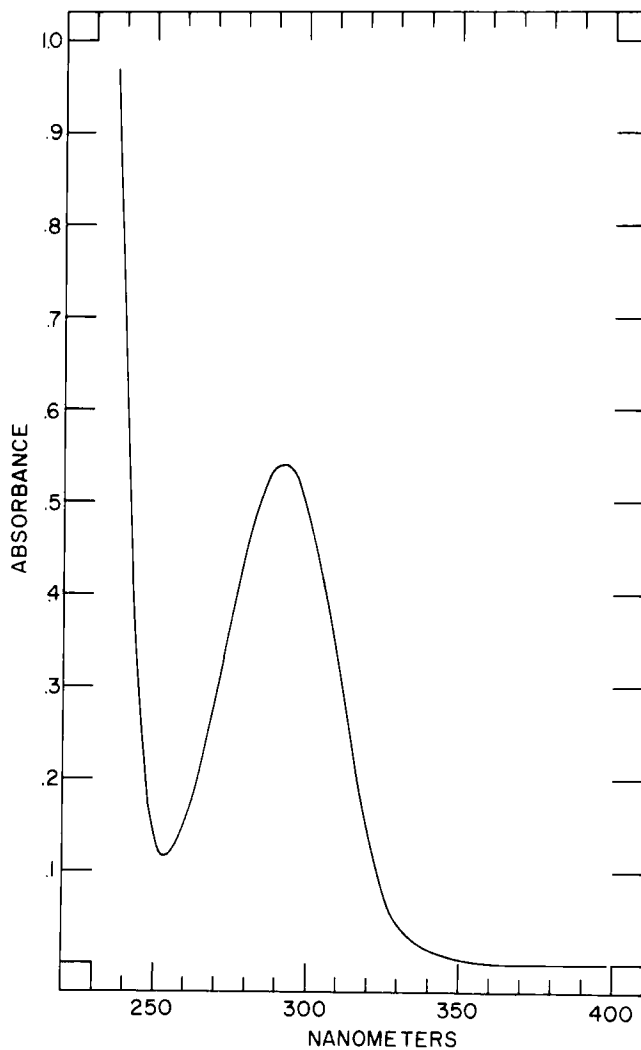
2.3 Ultraviolet Spectrum (UV)

When the UV spectrum of methyprylon in 2-propanol was scanned between 400 and 220 nm, one maximum and one minimum were observed. The maximum is located at 294 nm ($\epsilon = 33$) and the minimum at 353 nm. The spectrum shown in Figure 3 was obtained from a solution of 300.1 mg of methyprylon/100 ml of 2-propanol (3).

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Figure 3

Ultraviolet Spectrum of Methypnylon



2.4 Fluorescence Spectrum

The excitation and emission spectra for methypylon (1 mg/ml methanol) are shown in Figure 4 (4). The excitation spectrum exhibits a broad maximum at about 302 nm and a shoulder about 239 nm. The emission spectrum has one maximum about 396 nm.

2.5 Mass Spectrum

The mass spectrum of methypylon was obtained using a CEC 21-110 mass spectrometer with an ionizing energy of 70 eV. The output from the mass spectrometer was analyzed and presented in the form of a bar graph, shown in Figure 5, by a Varian 100 MS dedicated computer system which calculates relative peak intensities by comparing each peak to the most intense peak in the spectrum (5).

The weak peak at m/e 183 is due to the molecular ion. The base peak occurs at m/e 155 and is due to the loss of CO and/or C_2H_4 from the parent. The remaining peaks are compatible with the known fragmentation pattern of methypylon (5).

2.6 Optical Rotation

Methypylon contains an assymetric center at the 5-carbon in the ring. However, the racemic mixture is obtained from the synthesis and no optical activity is exhibited.

2.7 Melting Range

The melting range reported in the N.F. XIII for methypylon is 74° to 77.5° using the class I procedure (6).

2.8 Differential Scanning Calorimetry (DSC)

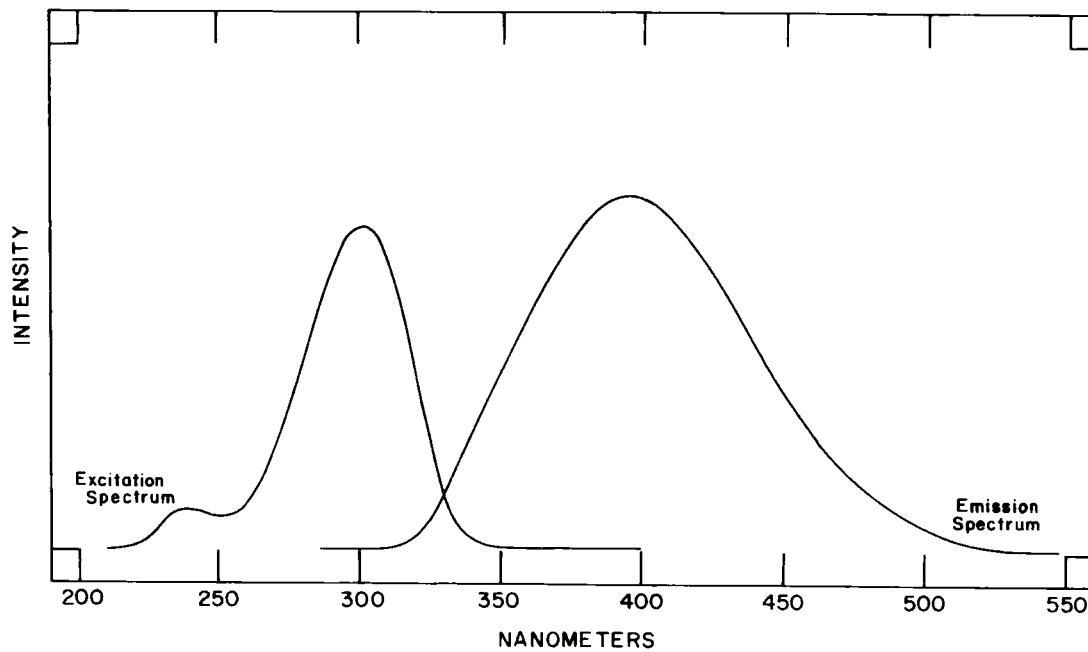
The DSC scan for methypylon is shown in Figure 6. A melting endotherm was observed at 76.1°C when the temperature program was $10^{\circ}/\text{minute}$. The ΔH_f was found to be 4.1 kcal/mole (7).

2.9 Thermogravimetric Analysis (TGA)

The TGA performed on methypylon exhibited no loss of weight from ambient to 115°C . One continuous weight loss was observed from 115° to 260°C which accounted for essentially all of the sample (7).

Figure 4

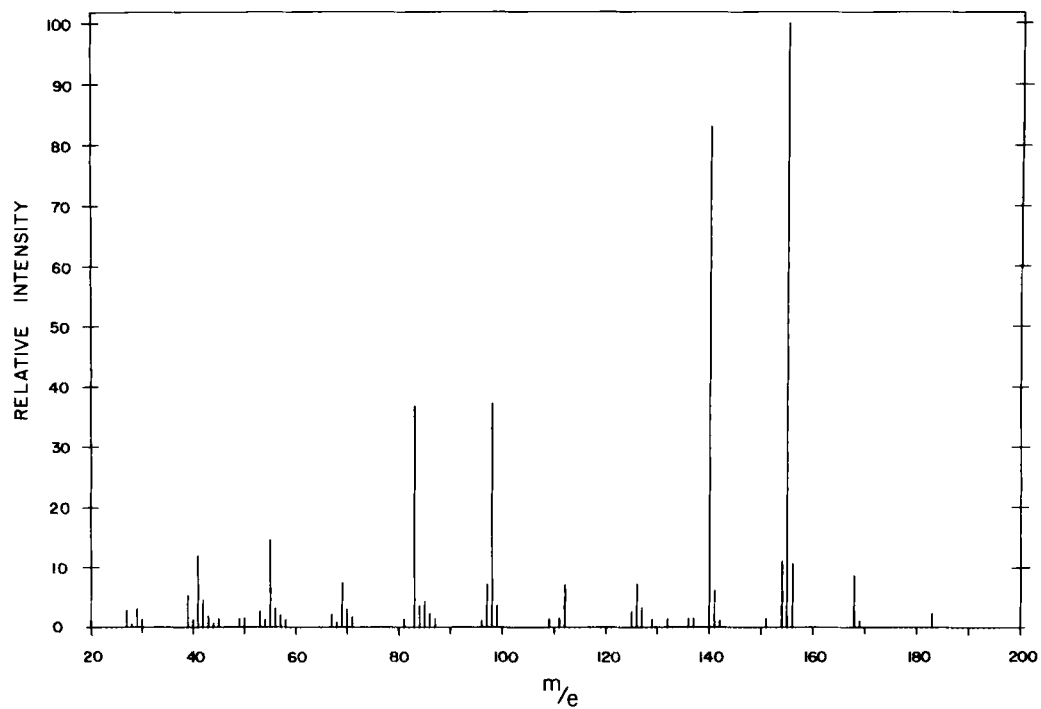
Fluorescence Spectra of Methyprylon



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Figure 5

Mass Spectrum of Methypylon

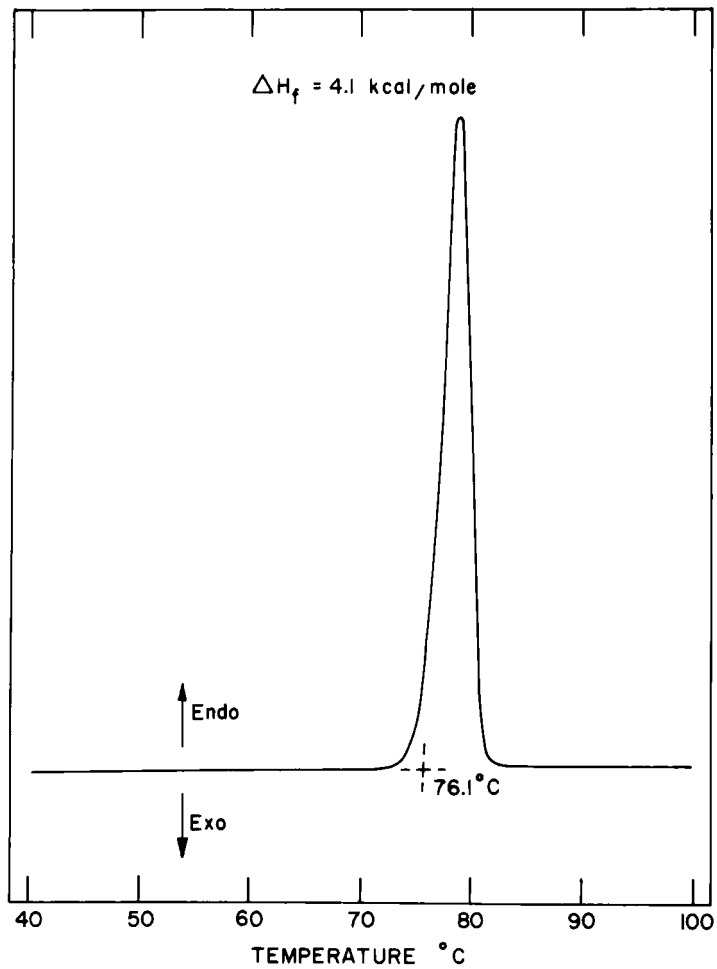


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Figure 6

DSC Scan for Methyprylon



2.10 Solubility

The solubility data for methypylon obtained at 25°C are presented in Table II (8).

Table II

Solubility of Methypylon in Different Solvents

<u>Solvent</u>	<u>Solubility (mg/ml)</u>
3A alcohol	$>5.0 \times 10^2$
benzene	$>5.0 \times 10^2$
chloroform	$>5.0 \times 10^2$
95% ethanol	$>5.0 \times 10^2$
ethyl ether	2.7×10^2
methanol	$>5.0 \times 10^2$
petroleum ether (30°-60°)	1.7×10
2-propanol	$>5.0 \times 10^2$
water	7.6×10

2.11 X-ray Crystal Properties

The x-ray powder diffraction pattern of methypylon is presented in Table III (9). The instrumental conditions are given below.

Instrument Conditions:

General Electric Model XRD-6 Spectrogoniometer

Generator:	50 KV-12-1/2 MA
Tube target:	Copper
Radiation:	Cu K_{α} = 1.542 Å
	0.1° Detector slit
	M.R. Soller slit
	3° Beam slit
	0.0007 inch Ni filter
	4° take off angle
Goniometer:	Scan at 0.2° 2θ per minute
Detector:	Amplifier gain - 16 course, 8.7 fine
	Sealed proportional counter tube and DC voltage at plateau

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Pulse height selection E_L - 5
volts; E_u - out
Rate meter T.C.4
2000 C/S full scale
Recorder: Chart speed - 1 inch per 5
minutes

Samples prepared by grinding at room temperature.

Table III

X-ray Powder Diffraction Pattern of Methyprylon

2θ	$d(\text{\AA})^*$	I/I_o^{**}	2θ	$d(\text{\AA})^*$	I/I_o^{**}
10.00	8.85	13	35.02	2.56	8
13.78	6.43	100	35.36	2.54	3
14.64	6.05	53	35.77	2.51	4
15.62	5.67	7	36.22	2.48	1
16.29	5.44	50	38.18	2.36	3
18.90	4.70	7	38.88	2.32	1
19.54	4.54	3	39.10	2.31	1
20.24	4.39	28	40.10	2.25	8
20.72	4.29	6	40.49	2.23	6
21.96	4.05	11	40.82	2.21	4
22.00	4.04	11	41.98	2.15	2
24.00	3.71	12	42.32	2.14	2
24.55	3.63	4	42.72	2.12	2
25.32	3.52	29	43.02	2.10	1
26.14	3.41	17	43.12	2.10	2
26.88	3.32	23	44.06	2.06	2
27.36	3.26	2	44.52	2.04	2
27.86	3.20	14	44.66	2.03	2
28.68	3.11	3	44.80	2.02	2
28.78	3.10	3	45.18	2.00	2
28.90	3.09	3	46.70	1.95	1
29.51	3.03	12	48.34	1.88	2
30.42	2.94	18	48.58	1.87	1
31.60	2.83	4	50.00	1.82	1
33.14	2.70	6	50.30	1.81	2
34.32	2.61	4			

* d - (interplanar distance) $\frac{n\lambda}{2 \sin \theta}$
 ** I/I_o = relative intensity
 (based on highest intensity of 1.00)

2.12 Dissociation Constant

The pK_a for methyprylon has been determined spectrophotometrically to be approximately 12.0 (10).

3. Synthesis

Methyprylon is prepared by the reaction scheme shown in Figure 7. 3,3-Diethyl-1,2,3,4-tetrahydro-2,4-pyridinedione, Presidon, is reacted with formaldehyde yielding 3,3-diethyl-5-hydroxymethyl-1,2,3,4-tetrahydro-2,4-pyridinedione (HMP) which is then hydrogenated using a Raney nickel catalyst to methyprylon (11).

4. Stability Degradation

When methyprylon is refluxed in acidic, neutral, or basic aqueous solutions in the presence of oxygen, it breaks down into several carboxylic acids; e.g. formic acid, acetic acid, propionic acid, and diethyl acetic acid. Traces of unidentified amines and ketones are also observed (12). When pure methyprylon is stored in well-closed, light resistant containers, the substance is quite stable.

5. Drug Metabolic Products

The major metabolic pathways of methyprylon in dogs and humans is shown in Figure 8 (13-18). Methyprylon as the intact drug as well as its 5,6-dehydrogenated metabolite are found in blood (13,14). In addition to the 5,6-dehydrogenated metabolite, several other metabolites due to oxidation are found in urine. These include the 6-oxy-metabolite from the direct oxidation of methyprylon and the oxidation of the methyl group to the alcohol and then to the acid in the 5,6-dehydrogenated metabolite (16,18).

6. Methods of Analysis

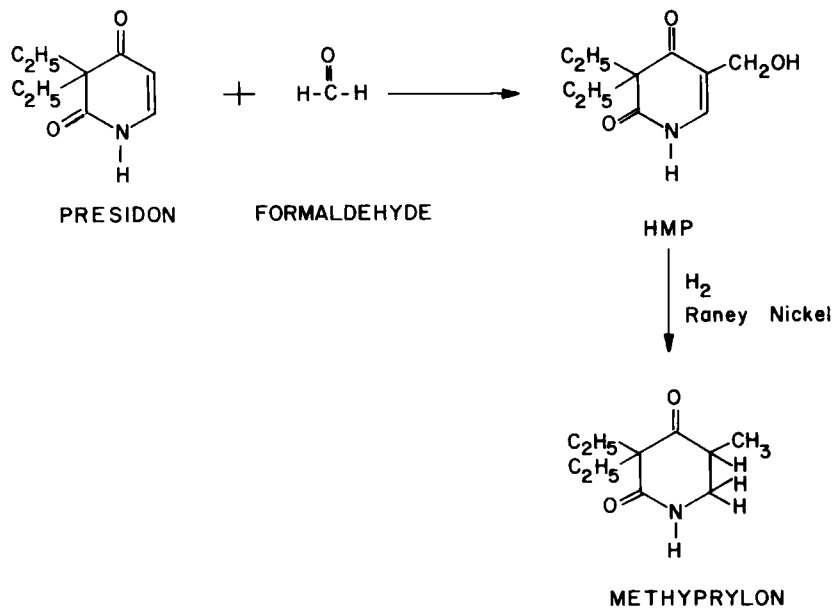
6.1 Elemental Analysis

The results from the elemental analysis are listed in Table IV (19).

<u>Element</u>	<u>Theory</u>	<u>% Found</u>
C	65.54	65.40
H	9.35	9.46
N	7.64	7.62

Figure 7

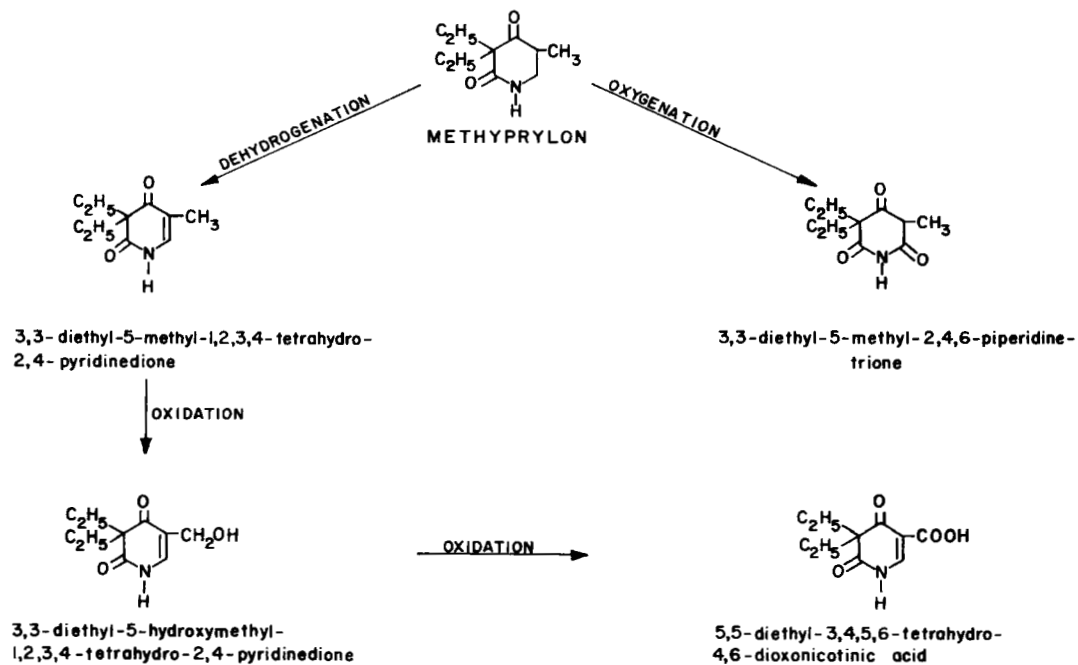
Synthesis of Methyprylon



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Figure 8

Metabolic Products of Methyprylon



6.2 Phase Solubility Analysis

Phase solubility analysis may be carried out using n-heptane as the solvent. A typical example for methyprylon is shown in Figure 9 which also lists the conditions under which the analysis was carried out (8).

6.3 Thin Layer Chromatographic Analysis (TLC)

A TLC system used to identify methyprylon from other hypnotics was published by Frahm et al. (20). This system utilized a Kieselgel G plate and a developing solvent of 2-propanol:chloroform:25% ammonium hydroxide (9:9:2). The R_f values were very sensitive to the amount of solvent saturation in the tank and therefore a reference standard was always run along with the sample for identification purposes.

A TLC system used to separate methyprylon from its metabolites and other similar compounds employs ethyl acetate as the developing solvent. The methyprylon sample, approximately 100 mcg, is spotted on a silica gel G plate and subjected to ascending chromatography until the solvent front has moved at least 15 cm. The plate is air dried and sprayed with freshly prepared potassium ferricyanide (1.6 gm $K_3Fe(CN)_6$ in 100 ml 2N KOH) and examined immediately under long-wavelength ultraviolet radiation. Methyprylon has an R_f of 0.40 in this system (21).

6.4 Gas-Liquid Chromatographic Analysis (GLC)

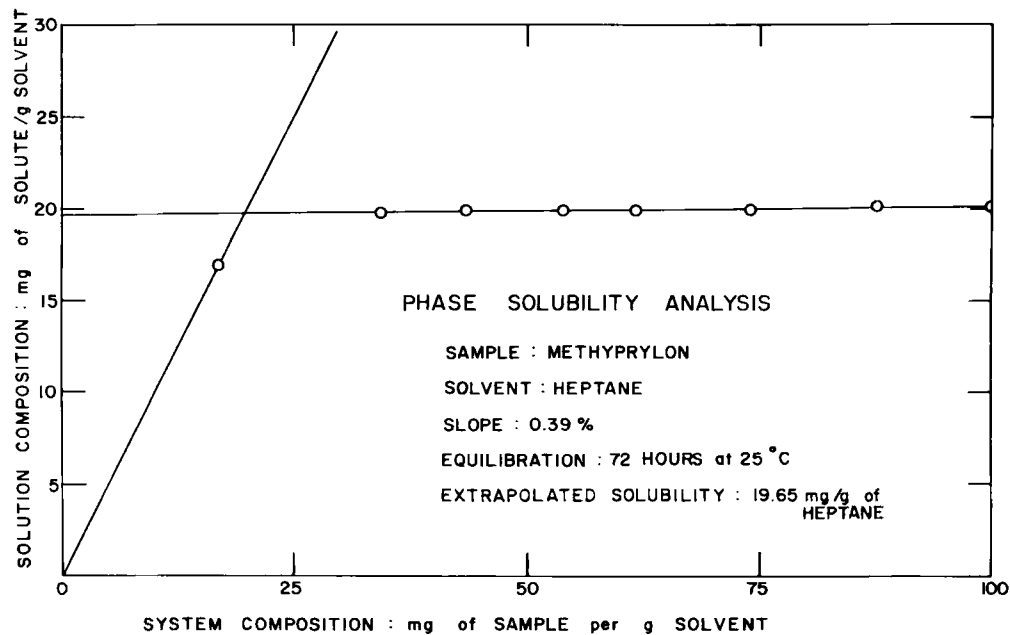
The following GLC method is useful for the separation and quantitation of methyprylon in the presence of its metabolites and similar structured compounds. The pertinent experimental conditions as well as the retention time for methyprylon are given in Table V (22).

Table V

GLC Method for Methyprylon

Column:	6 ft, 1/8 in O.D., stainless steel tubing
Support:	Diatoport S (60-80 mesh)
Stationary Phase:	8% Craig Polyester Succinate (BDS)
Detector:	Hydrogen Flame Ionization

Figure 9



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Temperature °C -
Injection Port: 210
Column: 180
Detector: 250
Flow Rate: 40 cc/minute of Nitrogen
Quantities Injected: 10-100 µg of methyprylon in
95% ethanol
Retention Times: 11 minutes

6.5 Direct Spectrophotometric Analysis

Direct spectrophotometric analysis is rarely used because of the very low molar absorptivity of methyprylon. However, in the absence of any interfering species, the maximum at 294 nm (in 2-propanol) could be used for direct spectrophotometric analysis.

6.6 Colorimetric Analysis

Methyprylon undergoes enolization in the presence of alkali thus permitting the use of the Folin Ciocalteu reagent to form the blue colored complex (23). This method is utilized for the determination of methyprylon present in blood or plasma. After appropriate extraction or other separation, the Folin Ciocalteu reagent is added to a basic solution of the methyprylon and the absorbance of the blue complex is measured at the maximum about 700 nm (14). The sensitivity limit is about 5 µg/ml of blood or plasma.

6.7 Titrimetric Analysis

The potentiometric titration of a basic, aqueous solution of methyprylon with 0.1N potassium ferricyanide is the method of choice for the analysis of methyprylon in the bulk form as well as in capsules and tablets (24). The end point is determined potentiometrically by the use of a calomel-platinum electrode system. Each ml of 0.1N potassium ferricyanide is equivalent to 9.162 mg of methyprylon.

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PHENELZINE SULFATE

Robert E. Daly

Reviewed by L. Chafetz

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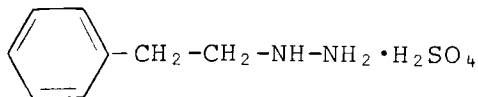
Analytical Profile - Phenelzine Sulfate

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1. Description

1.1 Name, Formula, Molecular Weight

Phenelzine Sulfate is β -phenylethylhydrazine dihydrogen sulfate. It is also known as phenethylhydrazine sulfate.



$C_8H_{12}N_2 \cdot H_2SO_4$

Mol. Wt.: 234.28

1.2 Appearance, Color, Odor

White to yellowish white crystalline powder having a characteristic odor.

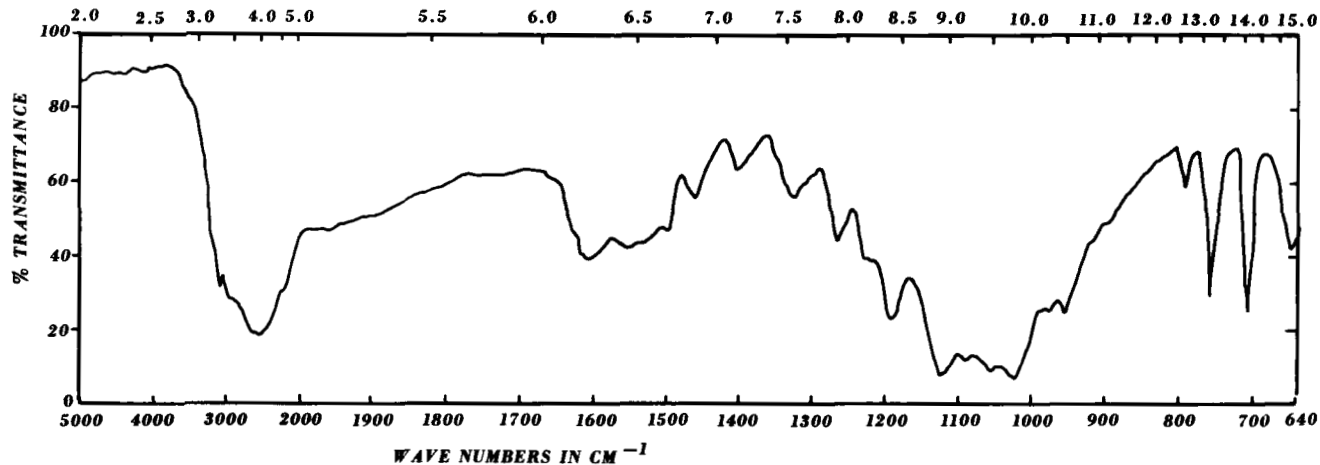
2. Physical Properties

2.1 Infrared Spectrum

The infrared spectrum (Figure 1) of phenelzine sulfate was determined as a KBr pellet in a Perkin-Elmer model 621 spectrophotometer. The spectrum obtained is identical to that of phenelzine sulfate which appears as Sadtler infrared spectrum #24825. The broad band at about 2500 cm^{-1} may be attributed to the hydrazine salt. The bands at 758 cm^{-1} and 705 cm^{-1} may be attributed to the monosubstituted benzene moiety.

2.2 Nuclear Magnetic Resonance Spectrum

The NMR spectrum of phenelzine sulfate was determined in a Varian A-60 instrument employing DMSO as the solvent (Figure 2). The spectrum displayed singlets at δ 7.75 (hydrogen bonded to nitrogen) and at δ 7.2 (aromatic). Other absorption was centered around δ 3.0 (methylene bonded to methylene and NH) and δ 2.5 (methylene bonded to methylene and phenyl). Deuterium exchange (Figure 3) was performed on the sample. The absorption band at δ 7.75 disappeared verifying the assignment of this band to hydrogen bonded to nitrogen.



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Figure 1 - Infrared Spectrum of Phenelzine Sulfate.

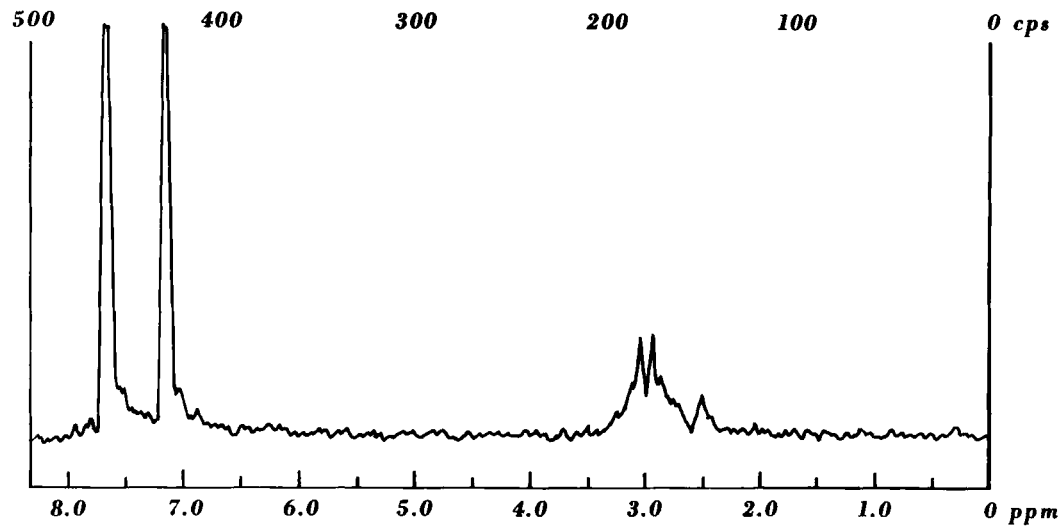


Figure 2 - NMR Spectra of Phenelzine Sulfate

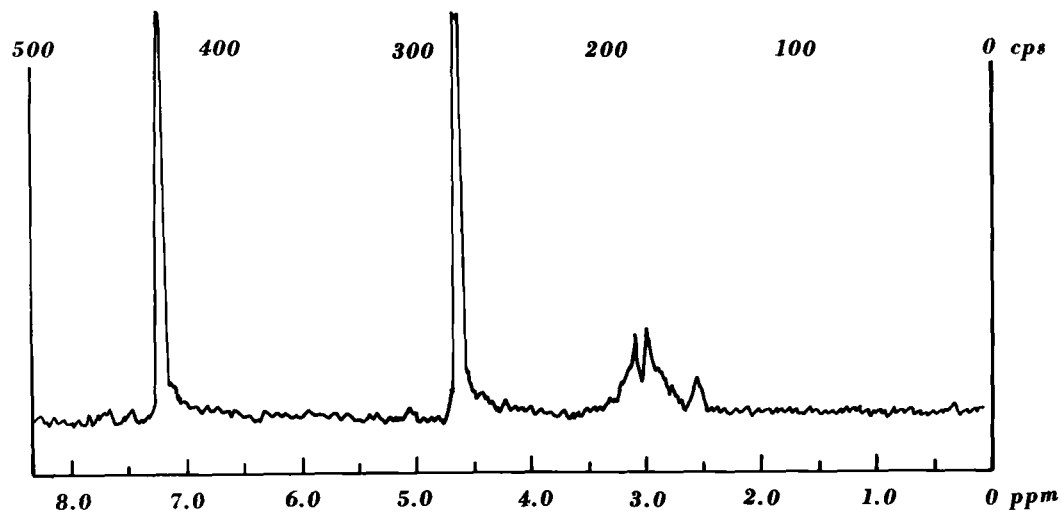


Figure 3 - NMR Spectra of Phenelzine Sulfate - Deuterium Exchange

2.3 Mass Spectrum

A mass spectrum of phenelzine sulfate could not be obtained despite several attempts. An AEI 902 was employed with an ionizing voltage of 70 ev and at a temperature of 100°C. On running the material directly in the probe, a spectrum which showed large amounts of sulfur dioxide resulted. Attempts at running the free base proved fruitless also. The sample was introduced into the mass spectrometer through the heated inlet but thermal decomposition occurred and no spectrum was obtained.

2.4 Ultraviolet Spectrum

Rajeswaran and Kirk (1) reported λ max (ϵ 1862) of 258 nm. and λ min of 228 nm. in 50% ethanol.

Phenelzine sulfate (0.0541% in 50% ethanol) when scanned in a Cary 14 spectrophotometer between 350 and 220 nm. (Figure 4), exhibited three peaks at 252, 258 and 263 nm. with λ max at 258 nm. (α = 0.798 : ϵ 1869).

2.5 Differential Thermal Analysis

A differential thermogram has been obtained in a Dupont model 900 DTA Instrument employing a DSC cell. Two endotherms, one at 134°C. (crystalline change) and the second at 172°C. (melting) were observed (Figure 5). Heating rate was 10° C./minute. The determination was carried out in a nitrogen atmosphere. Alteration in the rate of heating would tend to shift the endotherms.

2.6 Thermogravimetric Analysis

A thermogravimetric analysis was performed (Figure 6) in a Dupont model 950 TGA Instrument. The measurement was performed under nitrogen sweep at a heating rate of 10° C./minute. No weight loss was noted until about 165° C. (initiation of melting). After this point, weight was rapidly lost. This information together with the DTA indicates that the

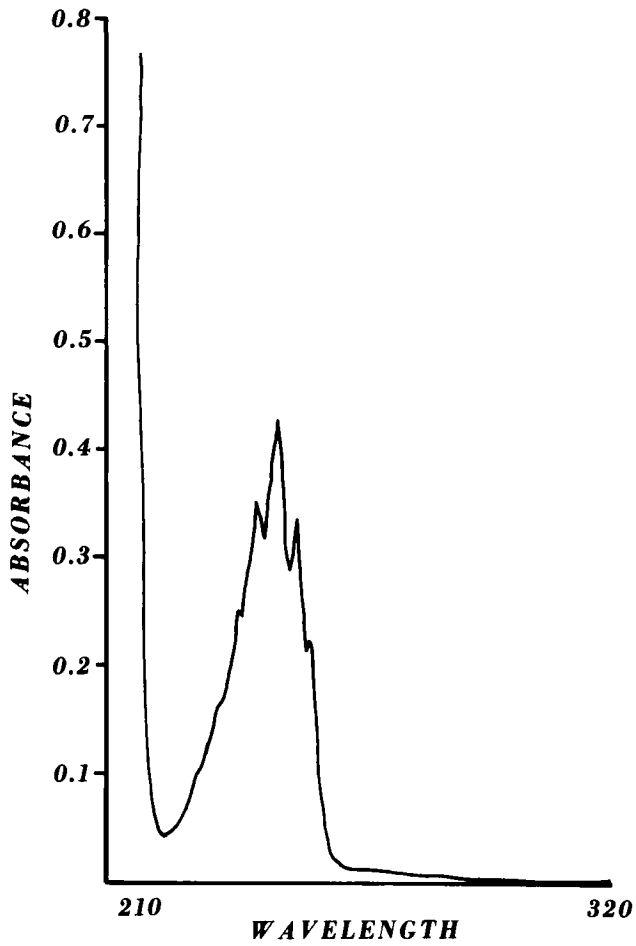


Figure 4 - UV Spectrum of Phenelzine Sulfate

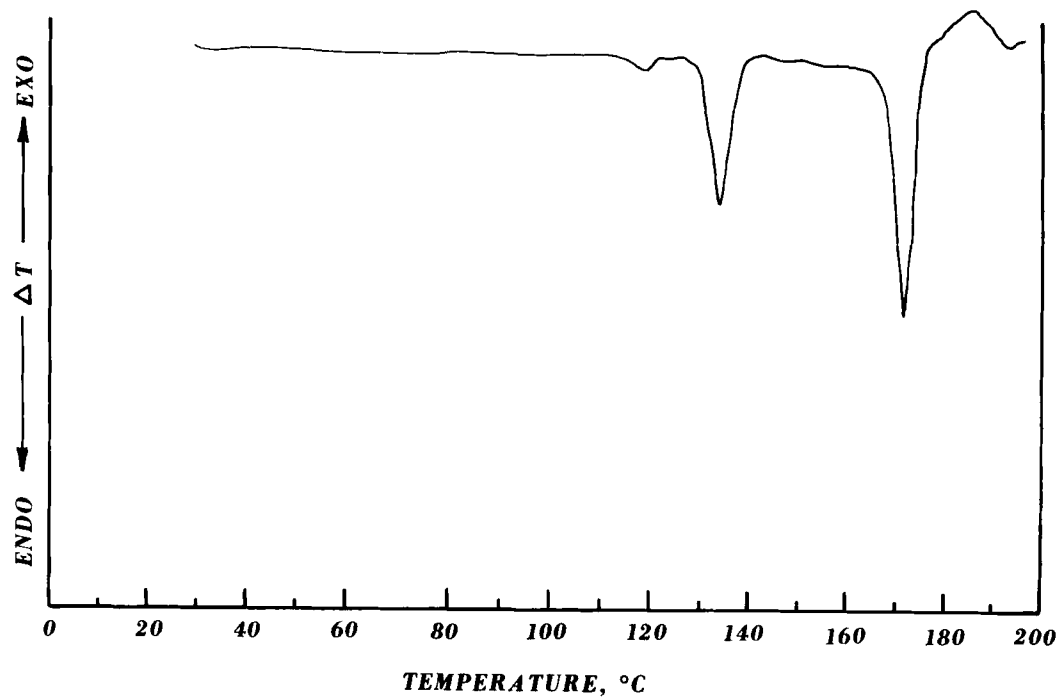


Figure 5 - Differential Thermogram of Phenelzine Sulfate

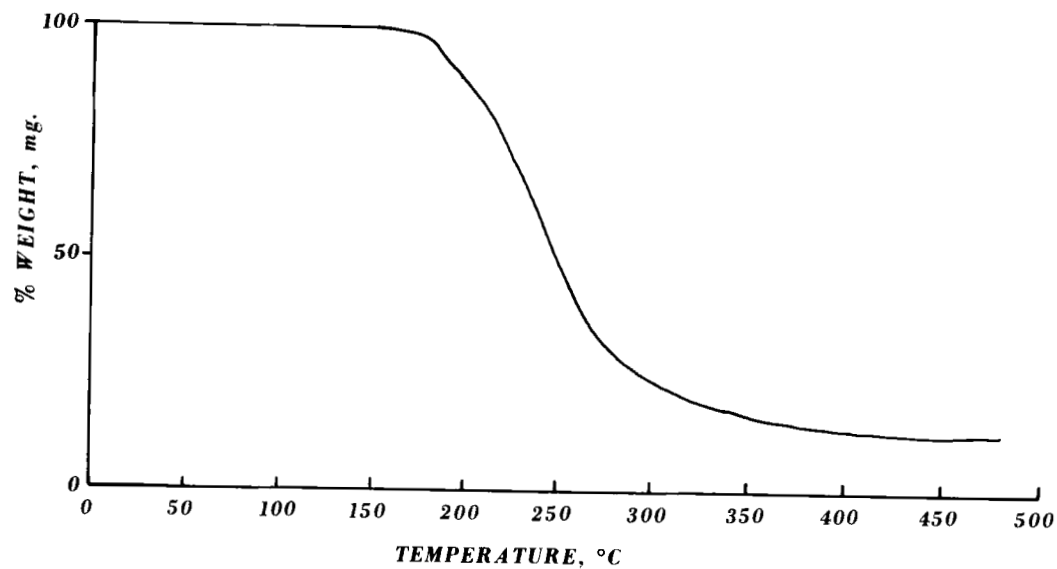


Figure 6 - Thermogravimetric Analysis of Phenelzine Sulfate

endotherm at 134° C. was due to a nondestructive alteration in the molecule (crystal change).

2.7 Melting Range

The melting range has been reported (2) to be 164 to 168° C.

2.8 Solubility

Phenelzine sulfate is soluble in water and practically insoluble in ethanol, chloroform and ether.

2.9 X-Ray Diffraction Powder Analysis

Rajeswaran and Kirk (1) have reported x-ray diffraction data for phenelzine sulfate. The powdered sample which had been appropriately mounted on a rotary specimen holder, was subjected to copper k-alpha radiation in a Norelco Geiger Counter X-Ray Spectrometer. The diffraction patterns were obtained over an arc of 45°. The instrument was calibrated over this range, using powdered quartz crystals, and the "d" values computed. Values are given in Table I.

Table I - "d" Distances Using
Copper K-Alpha Radiation

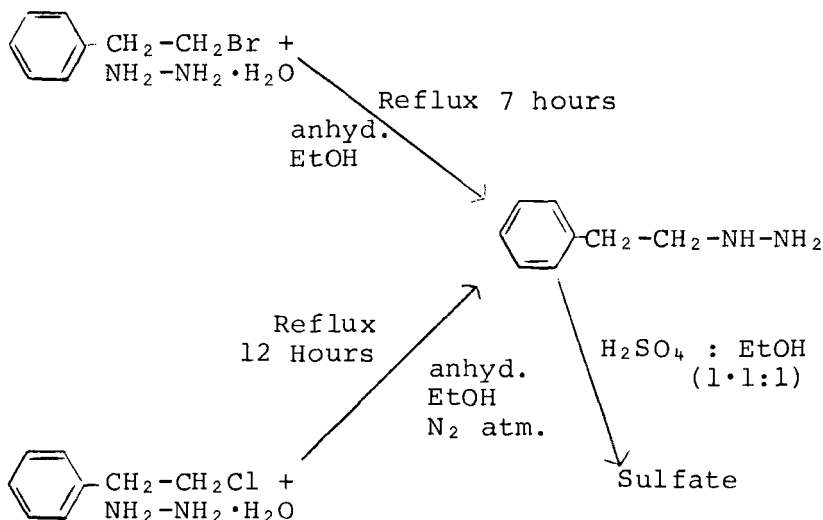
Distances - 18.9, 9.85, 6.51, 5.42, 4.89, 3.90,
3.24, 2.79 & 2.44

3. Synthesis

Biel (3) has synthesized phenelzine by reacting hydrazine hydrate and phenethyl bromide under reflux for 7 hours. A yield of 77% was obtained. Sacha and Malasnicki (4) condensed phenethyl chloride with hydrazine under reflux in anhydrous ethanol in a nitrogen atmosphere for 12 hours. The solution of the free base which was obtained after removal of hydrazine HCl by filtration was treated with sulfuric acid in anhydrous ethanol

(1.1/1 W/W). A yield of 21% of β -phenylethylhydrazine dihydrogen sulfate was obtained.

Biel, *et. al.* (5) has reported that aralkylation of hydrazine with primary aralkyl halides affords mono-(aralkyl)-hydrazines in yields of 60 to 75% provided a three to five fold molar excess of hydrazine hydrate is employed. Production of diphenylethylhydrazine has been reported (6) as a by-product in the reaction.



4. Stability

Phenelzine base is easily oxidized. Decomposition occurs when basified solutions are extracted, probably due to autooxidation of the base.

Schlitt, *et. al.* (7) have reported the autooxidation of phenelzine sulfate in pH 5.9 phosphate buffer. Autooxidation in pH 6.5, 0.1 M sodium chlorate at 37° C. has also been reported (8). Decomposition products were not characterized.

5. Metabolism

Drabner and Schwerd (9) identified unchanged

phenelzine and phenylacetylglutamine in the urine of humans who had received phenelzine sulfate.

Clineschmidt, *et. al.* (10, 11) studied the *in vitro* biotransformation as well as the *in vivo* metabolism of C^{14} -phenelzine in rats. Phenylacetic acid was identified as the major metabolite.

Fischer, *et. al.* (12) have described the effect of phenelzine on the excretion of β -phenethylamine.

6. Methods of Analysis

6.1 Spectrophotometric Analysis

6.11 Feigl (13) has reported the reduction of lithium and sodium molybdophosphotungstate solution by hydrazine derivatives. This reaction has been adapted to the determination of phenelzine sulfate raw material or tablets by Bose and Vijayvargiya (14). Reduction is carried out in alkaline solution with the production of a deep blue color which is measured at 650 nm.

6.12 A colorimetric method based on the formation of the hydrazone with vanillin has been devised (15). The reaction is carried out in 0.5 N methanolic hydrochloric acid. Absorbance of the yellow solution is read at 400 nm. Since phenelzine degrades by oxidative destruction of the hydrazine function, this method is stability indicating.

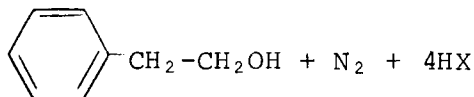
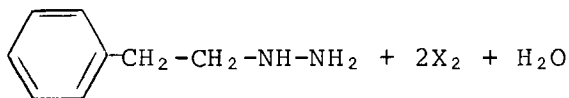
6.2 Polarography

The half wave potential ($E_{1/2}$ versus standard calomel electrode) of the acetone derivative of phenelzine was reported (7) as -1.33V in pH 5.9 phosphate buffer (0.1 M KH_2PO_4 : $Na_2HPO_4 \cdot 2H_2O$) employing a 0.01% gelatin solution as a maximum suppressor. The diffusion current constant (I_d) was approximately 2.5. Four electrons were transferred for the reduction of the hydrazone derivative. Waveheight was proportional to concentration in the range of 2.5×10^{-5} to 2.5×10^{-4} M in pH 5.9 phosphate buffer.

6.3 Titrimetry

Procedures based on the reaction of phenelzine sulfate with iodine in bicarbonate solution (2) and in sodium hydroxide (16) and with bromate and bromide in acid solution (16) with subsequent titration of excess reagent (iodine or bromide converted to iodine) in acid solution with thiosulfate have been reported.

Basically, the reaction involved may be illustrated as follows:



Radecka and Nigam (17) have reported the direct titration of phenelzine with N-bromosuccinimide in acidic solution employing methyl red as the indicator. However, their results show a 5% positive bias. This bias is attributed to allylic bromination by N-bromosuccinimide.

6.4 Chromatography

6.41 Gas Chromatography

Cardini, *et. al.* (18, 19) and McMartin and Street (20) have reported gas chromatographic methods which are reputed to be quantitative. However, no quantitative data are given. Conditions are reported in Table II.

6.42 Thin Layer Chromatography

Qualitative methods for the identification of psychotropic agents have been reported (21, 22, 23, 24, 25). R_f values are summarized in Table III.

6.5 Spot Tests

A variety of spot tests have been reported (26). These are summarized in Table V.

TABLE II - Parameters for Gas Chromatography

<u>Ref.</u>	<u>Column</u>	<u>Column Temp.</u>	<u>Carrier Gas</u>	<u>Flow Rate</u>	<u>Detector</u>	<u>Detector Temp.</u>	<u>Injector Temp.</u>
18	glass column 1.8 M x 2 mm.; 2% GE-SE 30 on Aeropak 30, 80- 100 mesh	program 70-250° at 10.4°/ minute	N ₂	50 ml./ min.	FID	220° C.	250° C.
19	Stainless steel column 1.2 M. x 2 mm.; 15% carbowax 20 M on Chromosorb W alkalized with 5% KOH	140° C.	He	40 ml./ min.	FID	---	250° C.
20	Stainless steel column 6' x 1/8"; 2% SE 30 + 0.1% tristearin on silanized acid washed Chromosorb W	140° C.	N ₂	30 ml./ min.	FID	---	---

TABLE III - Support, Solvent System and R_f
 Values for TLC of Phenelzine Sulfate

<u>Reference</u>	<u>Support</u>	<u>Solvent System</u>	<u>R_f (x 100)</u>
21	silica gel G	chloroform: methanol (8:2)	100
21	silica gel G	chloroform: acetone: diethylamine (5:4:1)	88
21	silica gel G	cyclohexane: chloroform: diethylamine (4:5:1)	62
22	silica gel G impregnated with 0.1 M NaOH	hexane:benzene: diethylamine (75:15:10)	51
22	silica gel G impregnated with 0.1 M NaOH	methanol	72
22	silica gel G impregnated with 0.1 M NaOH	acetone	75
22	silica gel G impregnated with 0.1 M NaHSO ₄	methanol	41

PHENELZINE SULFATE

TABLE III (cont.)

<u>Reference</u>	<u>Support</u>	<u>Solvent System</u>	<u>R_f (x 100)</u>
22	silica gel G impregnated with 0.1 M NaHSO ₄	95% ethanol	25
23	silica gel G activated at 110° C. for 30 minutes	chloroform: methanol (1:1)	37
23	silica gel G activated as above	chloroform: methanol: 20% ammonium hydroxide (2:1:1)	56
24	silica gel G activated at 100° C. for 1 hour	methanol:12 N ammonium hydroxide (100:1.5)	74
24	silica gel G impregnated with 0.1 N NaOH and activated as above	cyclohexane: diethylamine: benzene (75:20:15)	44
24	silica gel G impregnated and activated as above	acetone	65
24	silica gel G impregnated and activated as above	chloroform: methanol (90:10)	75

TABLE III (cont.)

<u>Reference</u>	<u>Support</u>	<u>Solvent System</u>	<u>R_f (x 100)</u>
24	silica gel G	benzene: ethanol: 12 N ammonium hydroxide (95:15:5)	50
25	silica gel G impregnated with 0.1 N KHSO ₄	95% ethanol	49
25	silica gel G impregnated with 0.1N NaOH	cyclohexane: benzene: diethylamine (75:15:10)	52
25	silica gel G impregnated with 0.1 N NaOH	methanol	72
25	silica gel G impregnated with 0.1 N NaOH	acetone	75
25	silica gel G impregnated with 0.1 N KHSO ₄	methanol	62
25	silica gel G impregnated with 0.1 N NaOH	methyl acetate	53

PHENELZINE SULFATE

TABLE III (cont.)

<u>Reference</u>	<u>Support</u>	<u>Solvent System</u>	<u>R_f (x 100)</u>
25	chromedia (Whatman #41) impregnated with 5% sodium dihydrogen citrate (some K ₂ HPO ₄ added to prevent tailing)	n-butanol: 5% citric acid	51

TABLE IV - Reagents and Reactions
For TLC Procedures

<u>Reference</u>	<u>Reagent</u>	<u>Reaction</u>
21	Folin-Ciocalteu's Reagent	blue violet with heating
21	5% phosphomolybdic acid in ethanol followed by exposure of the plate to ammonia vapor	blue
22	1% iodine in methanol	brown
23	50 ml. of 0.2% ninhydrin in methanol + 10 ml. of glacial acetic acid + 2 ml. of 2,4,6-trimethylpiperidine	rose orange
23	10% Ferric chloride; 1% fluorescein; ammonia	fluorescent blue
23	Saturated ammonium molybdate; saturated oxalic acid	celeste
24	Folin-Ciocalteu's Reagent	bluish at RT, blue when heated to 100° C.
24	Mandelin's Reagent	pink at RT, flesh at 100° C., fluorescent after heating
24	Cinnamaldehyde Reagent 5 ml. cinnamaldehyde + 95 ml. ethanol + 5 ml. conc. HCl	yellow at RT

PHENELZINE SULFATE

TABLE IV (cont.)

<u>Reference</u>	<u>Reagent</u>	<u>Reaction</u>
24	0.25 g. <i>p</i> -dimethyl-aminobenzaldehyde + 5 g. of 85% phosphoric acid + 20 ml. of water	lemon chromo after heating to 100 ° C. for 10 min. and then exposed to ammonia vapor
24	Furfural reagent sol'n A.) 1 ml. furfural in 99 ml. of acetone sol'n B.) 4 ml. of conc. H ₂ SO ₄ in 96 ml. of acetone	brown at 100° C.
25	Plate examined under shortwave UV light and then sprayed with the following sequence of reagents:	
	iodine	brown
	Dragendorff's	orange
	sodium nitrite	deep brown
	iodoplatinate until a color develops	variously colored

TABLE V - Spot Tests for Phenelzine Sulfate

<u>Reagent</u>	<u>Response</u>	
	<u>0.5 mg.</u>	<u>20 µg.</u>
Froehde's Reagent	blue* lt. blue	blue
Mandelin's Reagent	green blue* brown	green
Marquis' Reagent	---	faint orange
Mecke's Reagent	red orange* grey	pink
Reickard's Reagent	lt. blue	lt. blue
Flueckiger's Reagent	brown	---
Vitali's Reagent	a.) blue b.) orange	--- ---
Wasicky's Reagent	red orange	---

* = after warming

PHENELZINE SULFATE

TABLE VI - Composition of Spot Test Reagents

<u>Reagent</u>	<u>Composition</u>
Froehde's Reagent	0.5% aqueous ammonium molybdate
Mandelin's Reagent	0.5% aqueous ammonium vanadate
Marquis' Reagent	1 part 37% formaldehyde in 20 parts concentrated sulfuric acid, freshly prepared before use
Mecke's Reagent	0.5% aqueous selenious acid (H_2SeO_3)
Reickard's Reagent	1% aqueous sodium tungstate
Flueckiger's Reagent	0.5% solution of titanium dioxide (TiO_2) in concentrated sulfuric acid
Vitali's Reagent	a.) fuming nitric acid b.) 3% potassium hydroxide in ethanol
Wasicky's Reagent	10% solution of <i>p</i> -dimethylaminobenzaldehyde in glacial acetic acid

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PRIMIDONE

Raymond D. Daley

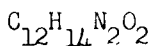
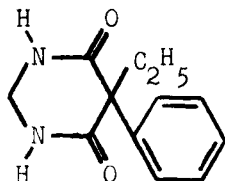
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1. Description

1.1 Name, Formula, Molecular Weight

Primidone is 5-ethylidihydro-5-phenyl-4,6(1H,5H)-pyrimidinedione.



Mol. Wt. 218.26

1.2 Appearance, Color, Odor, Taste

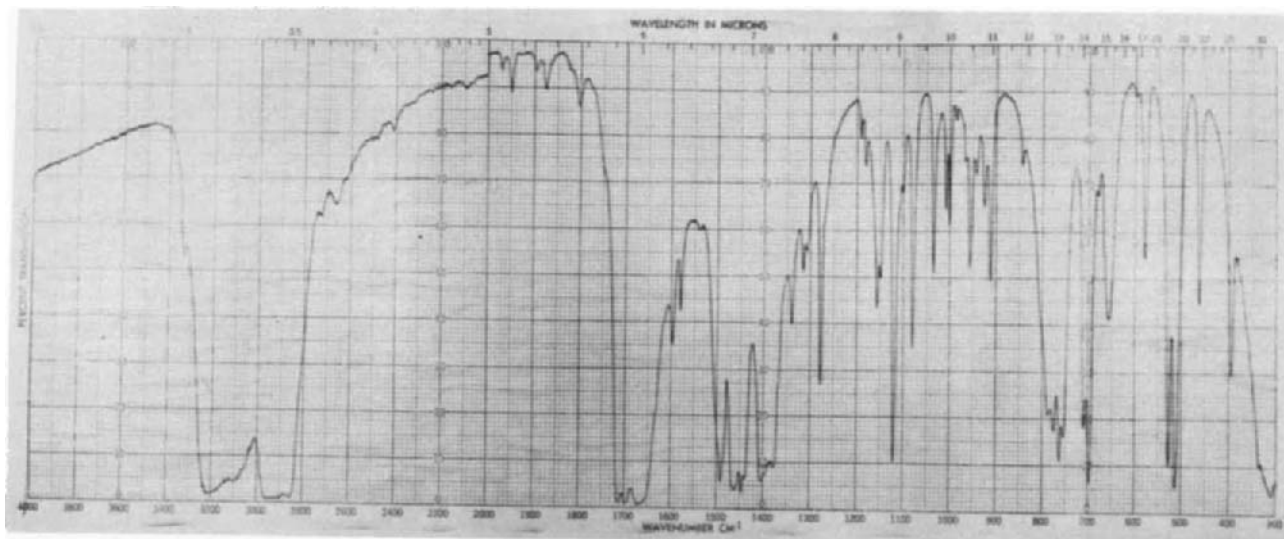
White, odorless powder, with a slightly bitter taste.

2. Physical Properties

2.1 Infrared Spectra

Infrared spectra of two crystal forms of primidone (here designated as Form I and Form II) are shown in Figures 1 and 2. The samples were prepared as mineral oil mulls between potassium bromide plates. A Beckman Model IR-12 instrument was used. Similar spectra are obtained with potassium bromide dispersions (1,2), but the pressing operation usually causes distortions of the absorption bands.

Some of the absorption bands may be assigned as follows: 3200 cm^{-1} region, bonded N-H stretch; 1700 cm^{-1} region, amide C=O; 1600, 1490, 760, 700, and 520 cm^{-1} , aromatic ring. The absorption in the 2900 cm^{-1} region and part of the absorption at 1460 and 1380 cm^{-1} is due to mineral oil.



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Fig. 1. Infrared spectrum of primidone, Ayerst Laboratories, Inc., house reference standard P24636B, Form I, mineral oil mull between KBr plates.

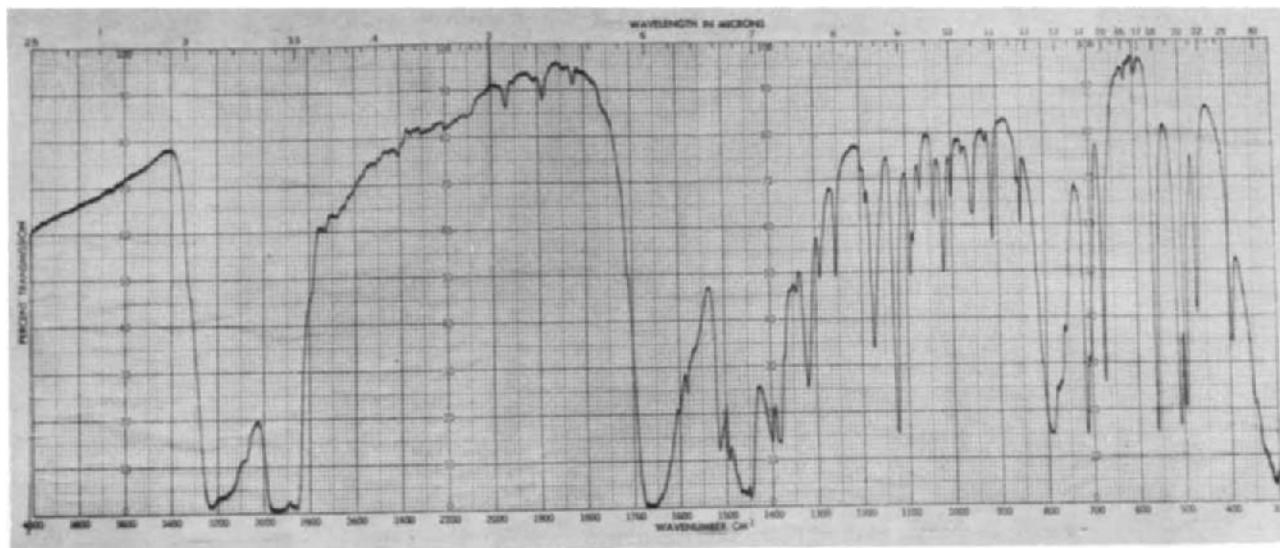


Fig. 2. Infrared spectrum of primidone, Form II, mineral oil mull between KBr plates.

2.2 Nuclear Magnetic Resonance Spectra

Figure 3 shows the proton magnetic resonance spectrum of primidone. This spectrum was obtained on a d₆-dimethylsulfoxide solution, using a Varian HA-100D instrument, with a tetramethylsilane reference. The assignment of the spectrum is shown in Table I (3).

The resonances at 3.96 and 4.12 ppm are due to the fragment NH-CH₂-NH in which the methylene protons have different chemical shifts and thus show geminal coupling, and in which one of the methylene protons (probably the equatorial) is coupled to both the NH protons and is thus a doublet of triplets. The system can be described as ABX₂ (3).

TABLE I

Proton Magnetic Resonance Spectrum Peak Assignments (3)

<u>PPM</u>	<u>Relative Intensity</u>	<u>Multiplicity</u>	<u>Assignment</u>
0.84	3	triplet	methyl of ethyl group
1.99	2	quartet	methylene of ethyl group
2.50	-	quintet	residual d ₅ -DMSO in solvent
3.35	-	singlet	water in solvent
3.96	1	doublet	one of the ring methylene protons
4.12	1	doublet of triplets	the other ring methylene proton
7.30	5	multiplet	phenyl group
8.64	2	doublet	NH protons

2.3 Ultraviolet Spectra

Figure 4 shows the ultraviolet absorption spectrum of primidone (Ayerst Laboratories, Inc., house standard P24636 B). The sample was dissolved in methanol (86.6 mg/100 ml) and scanned vs. methanol. Scans of

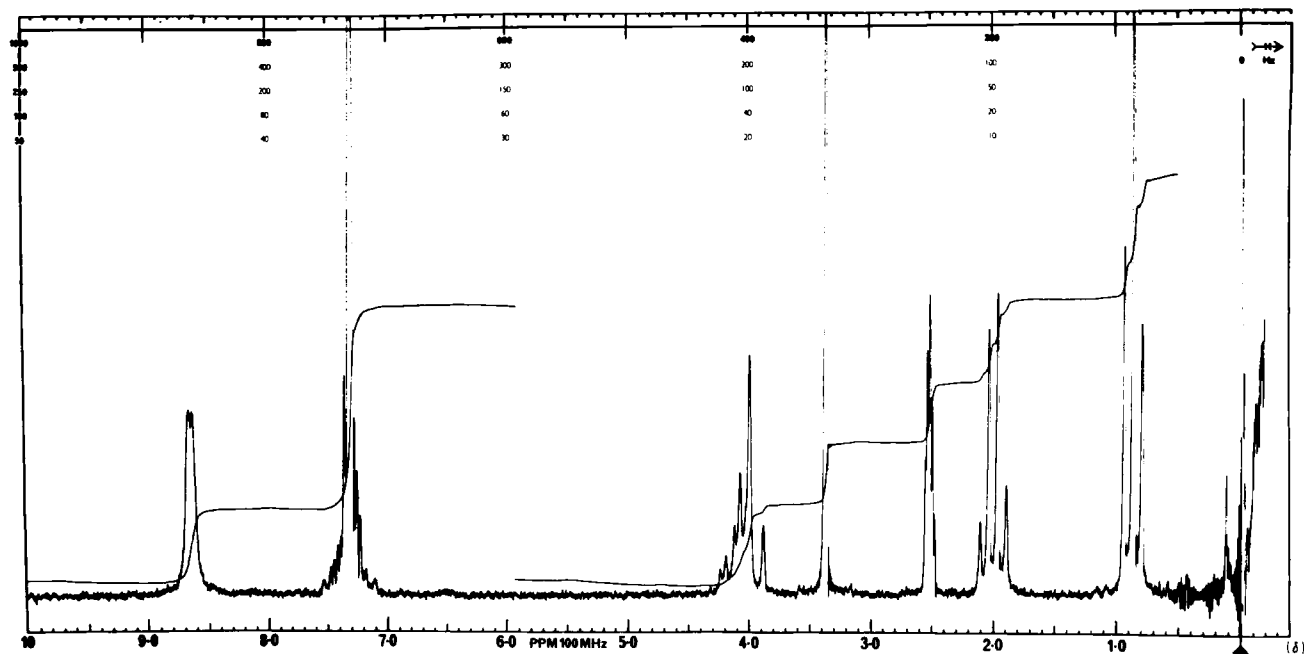


Fig. 3. Nuclear magnetic resonance spectrum of primidone in d_6 -dimethylsulfoxide, tetramethylsilane reference, courtesy of Dr. G. R. Bedford (3).

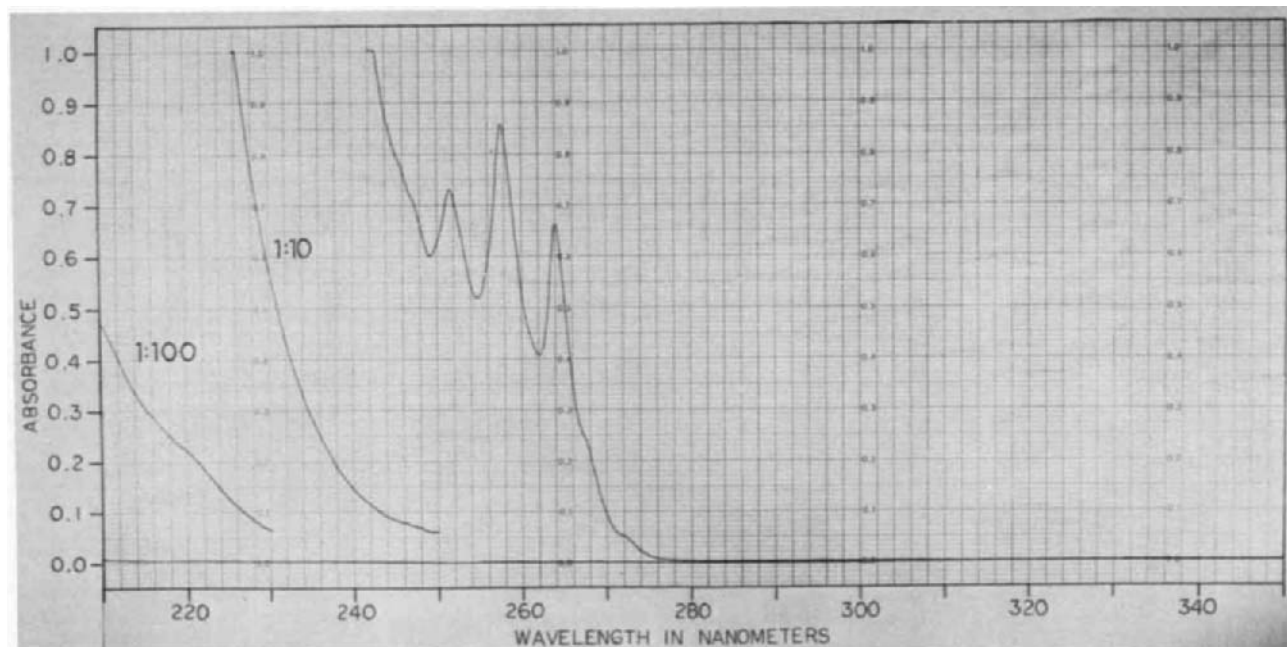


Fig. 4. Ultraviolet spectrum of primidone, Ayerst Laboratories, Inc., house reference standard P24636B, 86.6 mg/100 ml and 10- and 100-fold dilutions, in methanol, 1 cm cells.

10- and 100-fold dilutions of this solution are also shown. A Cary Model 14 spectrophotometer was used. Maxima at 264, 258, and 252 nanometers have absorptivities of 167, 216, and 183 liters per mole cm, respectively, in reasonable agreement with previously published data (1,4). There are no other maxima above 210 nanometers.

The absorption bands in the 260 nanometer region are caused by the phenyl group, while the absorption at shorter wavelengths is due to a combination of the phenyl and amide group absorptions.

2.4 Mass Spectra

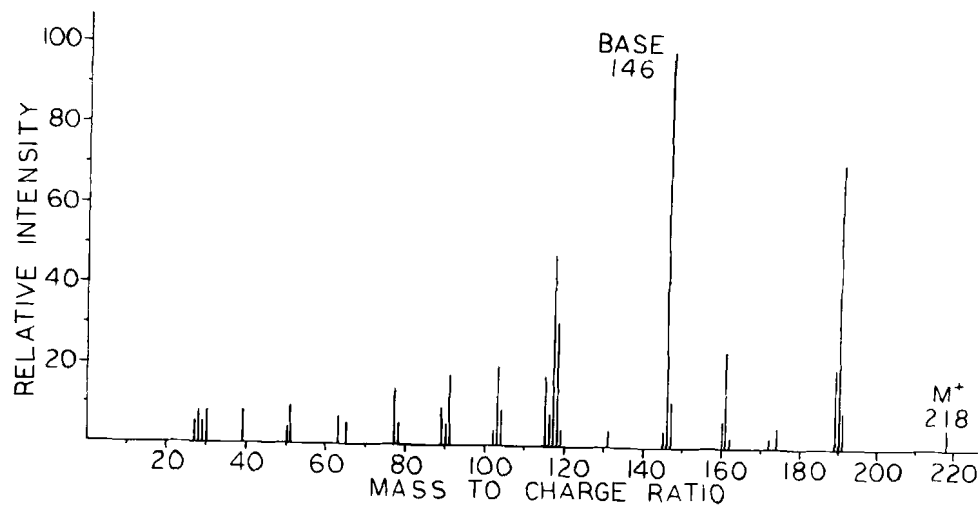
Figure 5 shows the low resolution mass spectrum of primidone. This data was obtained on a Perkin-Elmer/Hitachi RMU-6E mass spectrometer (3).

The spectrum matches that measured and interpreted by Locock and Coutts (5). The molecular ion $C_{12}H_{14}N_2O_2$ is at m/e 218. Loss of ethylene yields m/e 190 ($C_{10}H_{10}N_2O_2$), and loss of an ethyl radical yields m/e 189 ($C_{10}H_9N_2O_2$). Loss of CH_2NH from m/e 190 yields m/e 161 ($C_9H_7NO_2$). Loss of $C_2H_4N_2O$ from the molecular ion yields m/e 146 ($C_{10}H_{10}O$), the most abundant fragment. Loss of CO from m/e 146 yields m/e 118 (C_9H_{10}), which loses H and H_2 successively to form m/e 117 and m/e 115 (5).

At high resolution, m/e 161 is reported to be a doublet of compositions $C_9H_7NO_2$ and $C_{10}H_{11}NO$, with relative abundances 5:1 respectively; the latter, less abundant, ion can be derived from the molecular ion by loss of C_2H_3NO (6).

2.5 Crystal Properties

X-ray powder diffraction patterns (7,8) and infrared spectra (see Section 2.1) indicate that primidone may crystallize in at least two different forms. One of these (here designated Form I) usually crystallizes when a solution of primidone in 95 percent ethanol is evaporated at room temperature. The second (here designated Form II) usually forms if primidone



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Fig. 5. Mass spectrum of primidone, data courtesy of Dr. G. R. Bedford (3).

crystallizes from hot water or from the melt.

The powder diffraction patterns of the two forms are given in Table II. These patterns were obtained with a Norelco diffractometer, using nickel-filtered copper $K\alpha$ radiation. The pattern of Form I is the same as that reported by Roy et al (8), while the pattern of Form II is similar to that reported by Penprase and Biles (7).

TABLE II

X-ray Powder Diffraction Patterns

Form I		Form II	
<u>d</u>	<u>I/I₀</u>	<u>d</u>	<u>I/I₀</u>
10.96	17	13.77	14
7.37	100	6.88	100
6.57	46	6.16	20
5.98	70	5.75	35
5.46	51	5.18	6
4.96	18	4.59	55
4.83	50	4.18	28
4.44	26	4.16	46
4.06	8	3.98	6
3.88	58	3.93	5
3.73	28	3.72	6
3.66	18	3.65	4
3.56	15	3.44	6
3.42	15	3.27	5
3.21	20	3.13	12
3.16	10	3.06	4
3.12	11	2.98	4
2.98	22	2.75	4
2.82	15	2.66	6
2.72	10	2.50	4
2.63	5		
2.61	5		
2.55	7		
2.35	4		
2.33	4		
2.26	6		
2.25	5		
2.19	8		

2.6 Melting Points

The following melting points have been reported:

280-281°C	(15)
281°C	(11, 12, 13)
281-282°C	(9, 10)
282°C	(16)
289°C	(7)

The value 289°C was reported by the same investigators who reported the Form II diffraction pattern (see Section 2.5) (7).

2.7 Solubility

The solubility of primidone at room temperature is as follows:

<u>Solvent</u>	<u>Approximate Solubility, mg/ml</u>
Methanol	5.8
Ethanol (95%)	6.2
Acetone	2.0
Water	0.5
Chloroform	0.1
Ether	0.1
Benzene	< 0.1
Petroleum ether	< 0.1

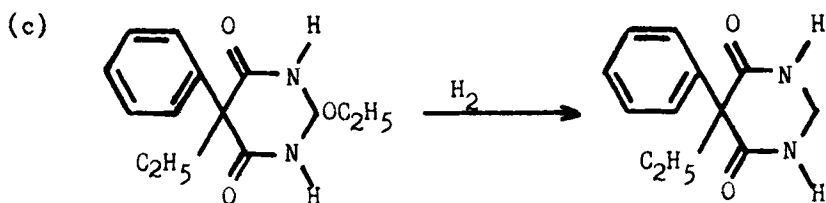
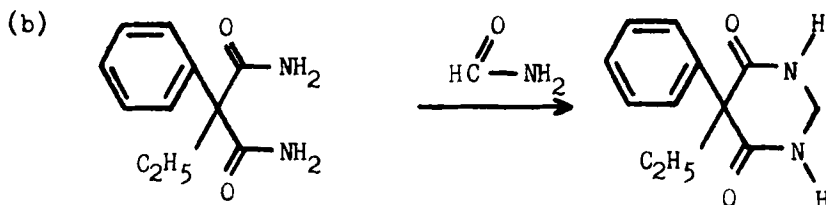
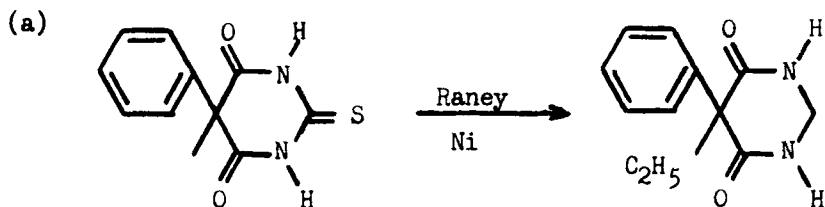
3. Synthesis

Primidone can be prepared by:

(a) Reductive desulfurization of 5-ethyl-5-phenylthiobarbituric acid with Raney nickel (9,12) or zinc and acid (9,21); (b) condensation of phenylethylmalondiamide with formamide (10), or formic or oxalic acid (14); (c) reduction of 2-ethoxy-5-phenyl-5-ethylhexahydro-4,6-pyrimidinedione with hydrogen or formic acid or formamide or zinc and acid (11,22); (d) hydrogenation of 2-methoxy-5-ethyltetrahydropyrimidine-4,6-dione (13); (e) electrolysis of

phenobarbital (12) or of the 2-imino or 2-cyanimino derivatives of 5-ethyl-5-phenylhexahydropyrimidine-4,6-dione (15); (f) reduction of 2-cyanimino-5-ethyl-5-phenylhexahydropyrimidine-4,6-dione with zinc and acid (15).

To help the reader visualize the processes above, (a), (b), and (c) may be depicted as follows:



4. Stability-Degradation

Primidone is quite stable, as might be predicted from the absence of reactive functional groups, low solubility, and high melting point. No reports of degradation under ordinary conditions were found, although it is metabolized (Section 5), and will react under drastic chemical conditions (Section 6).

5. Drug Metabolic Products

The reported metabolic products of primidone in various species are as follows:

<u>Metabolite</u>	<u>Species</u>	<u>References</u>
Phenylethylmalondiamide	man	(23,30)
	rabbit	(24)
	rat	(23)
Phenobarbital	man	(25,26,27,28,30,31)
	dog	(29,31)
	rat	(25,27)
	rabbit	(24)
	mouse	(29)
p-Hydroxyphenobarbital	rabbit	(24)
	dog	(31)
	man	(28,31)
p-Hydroxyphenobarbital glucuronide	rabbit	(24)

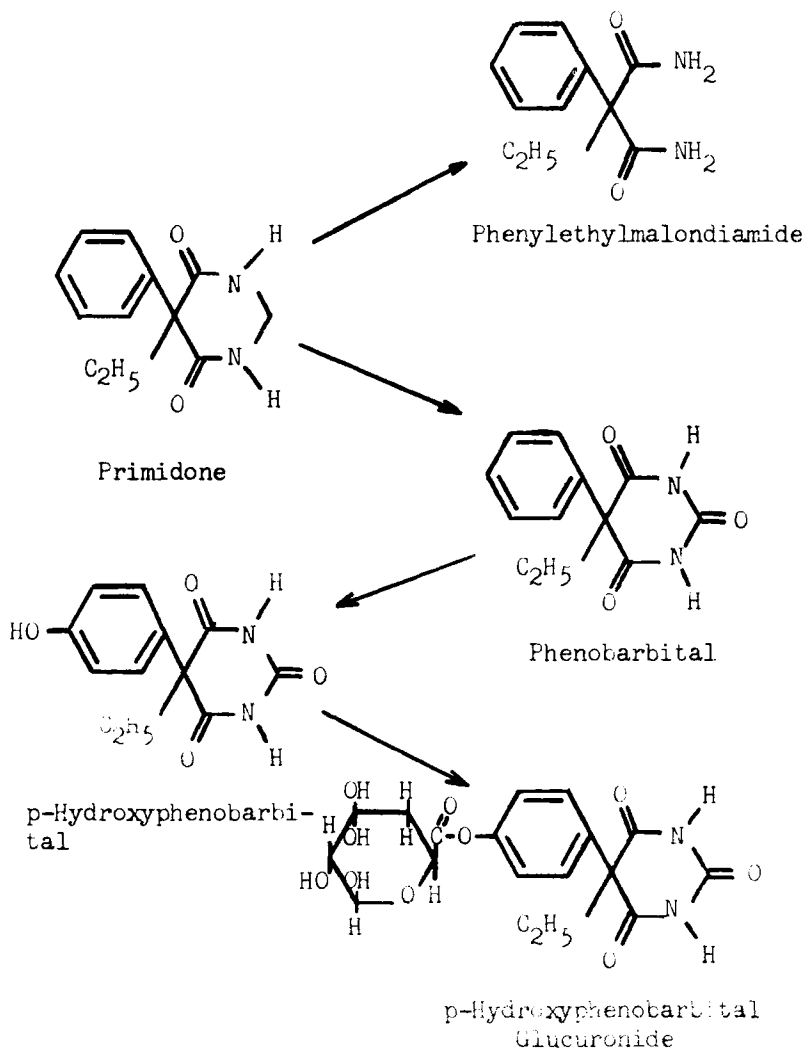
Structural formulas for these metabolites are shown in Figure 6.

6. Methods of Analysis

6.1 Elemental Analysis

Figure 6

Metabolism of Primidone



	<u>Theoretical (%)</u>	<u>Found (%) (12)</u>
Carbon	66.04	66.1
Hydrogen	6.47	6.5
Nitrogen	12.83	12.8
Oxygen	14.66	----

6.2 Chromatography

6.21 Gas Chromatography

Many investigators have used gas chromatography to analyze for primidone. Although the published work is concerned almost entirely with analyses for primidone in plasma or urine, many of the methods could be modified easily for analyses of pharmaceutical dosage forms.

A number of the systems used for gas chromatography are listed in Table III; additional information is given below.

Bogan and Smith (29) determined primidone and phenobarbital in blood and urine by gas chromatography. The samples were adjusted to pH 3 and extracted with chloroform. The drugs were further solvent partitioned, and the final solution in chloroform was evaporated to a small volume before injecting an aliquot into the gas chromatograph.

Pippenger and Gillen (32) determined primidone, phenobarbital, phenylethylmalondiamide, and a number of other anticonvulsants in plasma, urine, and cerebrospinal fluid. They extracted the sample with chloroform after buffering to pH 6.8. The chloroform extract was evaporated to dryness and the residue dissolved in a small amount of absolute ethanol for injection. Recoveries of known amounts of drugs added to the body fluids were 90 to 100 percent.

Street (62) identified primidone and other drugs by injecting a mixture of the drug and N,O-bis-(trimethylsilyl)-acetamide into the gas chromatograph. The silylating reagent was drawn into a 10 microliter syringe, then the sample solution was drawn into the same syringe, and the mixture was injected directly into the gas chromatograph. Trimethylsilyl derivatives were formed in the column. He used 4 microliters of 10% N,O-bis-(trimethylsilyl)-acetamide in acetone for 0.8 microliters of primidone solution containing 0.8 μ g of the drug.

Clarke (39) also lists a gas chromatographic system in which the retention time for primidone is 0.65 relative to codeine.

Evenson, Jones, and Darcey (33) extracted acidified serum with chloroform, evaporated the extract, took up the residue in a chloroform solution of dehydroisoandrosterone acetate internal standard, and analyzed the solution for both primidone and diphenylhydantoin by gas chromatography. The average recovery of primidone from serum was 98 percent. High grade chloroform was used to avoid extraneous peaks.

Furst et al (34) and Kupferberg (35) found that primidone could be converted to a methylated derivative in the gas chromatograph by injecting a solution containing primidone and trimethylanilinium hydroxide. Kupferberg used the reaction to determine primidone, phenobarbital, and diphenylhydantoin in plasma samples. The drugs were extracted with ethylene dichloride from samples buffered to pH 7.2, further solvent partitioned and mixed with cholestane internal standard, and finally mixed with trimethylanilinium hydroxide solution and injected into the gas chromatograph. Recovery of primidone from plasma or water was only 30 percent, probably because primidone was lost in the multiple solvent exchanges (35).

Van Meter, Buckmaster, and Shelly (41) used primidone as an internal standard in the gas chromatographic determination of phenobarbital and

diphenylhydantoin in plasma, and suggested that phenobarbital could be used as an internal standard in assaying primidone. Van Meter (42) recommended using mephentyoin as an internal standard for primidone assays. Other investigators have also used primidone as an internal standard in gas chromatography (43).

Gardner-Thorpe et al (36) determined primidone and a number of other antiepileptic drugs in plasma by gas chromatography. The drugs were extracted from acidified plasma samples with chloroform and injected without preparing derivatives. These investigators observed 30 percent recovery of primidone from plasma by their technique.

Proelss and Lohmann (37) developed a rapid gas chromatographic screening procedure for 40 drugs, including primidone, in serum. The drugs were separated into acidic, neutral, and basic fractions by solvent extraction. The three fractions were then analyzed by gas chromatography.

Kupferberg (38) reported that primidone formed a trimethylsilyl derivative with N,O-bis-(trimethylsilyl)-acetamide in pyridine. Primidone was extracted from plasma buffered to pH 7.2, using chloroform. After several solvent partitioning steps, the primidone was treated with the reagent for 20 minutes. The silylated derivative was stable for several hours. Recovery of primidone after the solvent partitioning steps was incomplete.

Toseland, Grove, and Berry (40) developed a routine procedure for gas chromatographic analyses of primidone and several other anticonvulsant drugs in blood. The drugs were extracted into chloroform and further solvent partitioned before chromatography. By doing double extractions, they were able to recover 58 to 67 percent of the primidone; they report not being able to reproduce the good recovery claimed by some other investigators. They preferred not to prepare derivatives, finding better reproducibility in day-to-day routine work with the underivatized drugs.

TABLE III

Gas Chromatographic Systems Used
for Primidone Analysis

Note: All systems listed used flame ionization detectors.

<u>Reference Number</u>	<u>Column</u>	<u>Carrier Gas</u>	<u>Column Temp °C</u>
(28)	6 ft. long x 1/8 in. O.D. stainless steel tubing 5% SE-30 on Aeropak 30 (100-120 mesh)	N ₂ 60 ml/min	180°
(32)	6 ft. long x 4 mm I.D. glass tubing, 1% HIEFF-8-BP on Gas Chrom Q, 100-120 mesh	N ₂ 18 psig	250°
(33)	125 cm long x 4 mm I.D. glass tubing, 3.8% W-98 on Diatoport S, 80-100 mesh	He 40 psig	215°
(35)	6 ft. long x 4 mm I.D. glass tubing, 3% OV-17 on Chromosorb W (HD)	N ₂ 80 ml/min	Pro- grammed from 160° to 275° at 10°/min
(36)	3 ft. long x 4 mm I.D., 1% CDMS (a) on Diatomite CQ	N ₂ 90 ml/min	250°
(38)	6 ft. long x 4 mm I.D. glass tubing, 3% OV-17 on 80-100 mesh Chromosorb W (HP)	N ₂ 80 ml/min	Pro- grammed from 160° to 250° at 10°/min

<u>Reference Number</u>	<u>Column</u>	<u>Carrier Gas</u>	<u>Column Temp °C</u>
(39)	5 ft. long x 4 mm I.D. glass tubing, 2.5% SE-30 on 80-100 mesh Chromosorb W AWHMDS	N ₂ 50 ml/min	225°
(40)	2 ft. long x 2 mm I.D. glass tubing, 2% SP-1000 on Gas Chrom W	He 50 ml/min	250°
(40)	5 ft. long x 1/4 in. I.D. glass tubing, 2% SP-1000 on Varaport	A 90 ml/min	250°
(44)	6 ft. long x 4 mm glass tubing, 1% OV-17 on HP Chromosorb G (80-100 mesh)	--	Pro- grammed from 190° to 230°
(42)	185 cm long x 3 mm I.D., 3% OV-1 on Chromosorb W (HP) (80-100 mesh)	N ₂ 30 ml/min	220°
(37)	180 cm long x 4 mm I.D. glass tubing, 3.5% XE-60 on Gas Chrom Q (100-120 mesh)	He 80 ml/min	240°

(a) CDMS is cyclohexane dimethanol succinate.

Gallagher and Baumel (44) determined primidone by gas chromatography after preparing a methylated derivative with N,O-bis-(trimethylsilyl)-trifluoroacetamide and dimethylformamide (5:1) at room temperature.

6.22 Thin Layer Chromatography

Huisman (45) extracted primidone and other anticonvulsant drugs from serum with chloroform/methyl acetate (60:40) after adding hydrochloric acid and sodium chloride. The extract was evaporated and the residue nitrated to form the nitrophenyl derivative of the drug. The nitrated material was chromatographed on fluorescent silica gel thin layer chromatographic plates with isobutyl alcohol/chloroform/25% ammonia (72:48:12); the developed plate was dried 10 minutes at 100°C and developed a second time. The spots were located under ultraviolet illumination; the nitrated primidone R_f was 0.76 to 0.80. The spots were scraped off, the nitrophenyl derivative reduced with zinc, the resulting amino group derivative diazotized with nitrous acid and coupled with α -naphthylethylenediamine, and the determination finished by measuring the absorbance at 550 nm. (The colorimetric procedure was adapted from that of Dill et al (50) for diphenylhydantoin.)

Fujimoto, Mason, and Murphy (24) extracted primidone from rabbit urine with chloroform after adding acetic acid. Chromatography was on silica gel impregnated glass fiber sheet, using 2,2,4-trimethylpentane/glacial acetic acid (5:1). The spots were detected by heating the sheet after spraying with concentrated sulfuric acid. The R_f of primidone was 0.7. The amounts of the drug were estimated from the size and intensity of the spots. The investigators reported 100 percent recovery of the drug from urine, and an accuracy of about 1 μ g primidone in 3 to 10 μ g spots.

Gardner-Thorpe, Parsonage, and Toothill (46) examined thin layer chromatographic systems for primidone and 20 other drugs. The drugs were extracted from plasma with chloroform after acidifying with hydrochloric acid. The chloroform extract was dried and

evaporated. The residue was dissolved and chromatographed on silica gel plates. Nine different solvent systems and ten detection systems were tested. The most sensitive detection system for primidone among those tested was exposure to iodine vapor, followed by spraying with a one percent aqueous solution of mercurous nitrate; this detected 5 μ g of primidone. (Sulfuric acid charring was not tested.) The R_f values for primidone in the solvent systems tested were as follows:

<u>Solvent</u>	<u>R_f</u>
1. Methanol/0.88 ammonia (38.5:1.5) (60 min equilibration)	.92
2. Di-n-butyl ether/1-butanol/acetic acid (80:40:10)	.49
3. Benzene/dioxane/0.88 ammonia (60:35:5)	.09
4. Ethanol/acetic acid/water (50:30:20)	1.00
5. Methanol/1-butanol (60:40)	.80
6. Chloroform/acetone (90:10)	.07
7. Chloroform/acetone (90:10) 60 min equilibration, filter paper lining tank	.05
8. Benzene/acetic acid (90:10)	.36
9. Benzene/dioxane/0.88 ammonia (75:20:5)	.03

Pippenger, Scott, and Gillen described (47) a rapid semi-quantitative method for determining primidone and several other drugs in plasma and urine. The drugs were extracted from acidified samples with chloroform. The extract was evaporated and the residue dissolved in a small amount of ethanol. Aliquots were spotted on three different silica gel plates, along with standards. The plates were developed in three different solvent systems and examined under short wavelength ultraviolet illumination. The lower limit of detection for primidone was 2 μ g. The solvents and R_f values for primidone were as follows:

<u>Solvent</u>	<u>R_f</u>
Chloroform/acetone (9:1)	.19
Carbon tetrachloride/acetone (7:3)	.26
Benzene/acetone (4:1)	.11

Yamamoto, Suzuki, and Okuo report (48) a sensitivity of one μg primidone by chromatographing on silica gel with chloroform/acetone (9:1). The R_f value was 0.06 and detection was with iodine vapor and one percent mercurous nitrate.

Garrettson and Dayton (49) described a thin layer chromatographic method in which the spots were scraped off the plate, the drug extracted with 20 percent alcohol in ether, the extract evaporated, and the drug determined colorimetrically by the method of Dill et al (50). (See method of Huisman above, in this Section.) Their method is about 10 times as sensitive as that of Huisman.

6.23 Paper Chromatography

Rusiecki, Henneberg, and Ostrowska (25) reported a paper chromatographic separation of primidone and phenobarbital. The drugs were extracted with chloroform from acidified blood, urine, or homogenized organs. The extracts were washed with pH 7.4 phosphate buffer and evaporated to dryness. The residue was dissolved in methanol and chromatographed on Whatman No. 1 paper impregnated with formamide. With ascending chromatography using the solvent system isopropyl alcohol/chloroform/25% ammonia (45:45:10), the R_f for primidone was 0.97-0.98, while that for phenobarbital was 0.67. Detection was by spraying with mercuric chloride and eosin in 95 percent ethanol. The minimum detectable amount of primidone was 50 μg .

6.3 Absorptiometric Methods

6.31 Ultraviolet Absorption

The ultraviolet absorption in the 250 nm region is the basis for a number of methods for determining primidone (1,4,17,18,39). The USP XVIII method (17) uses a 254 to 261 nm baseline in measuring the 257 nm peak absorbance; this will reduce interference from some ultraviolet absorbing materials, but others will have an absorption pattern similar to that of primidone. The method is therefore not specific enough for many

purposes. Furthermore, the absorptivity is not high, and this increases the probability of interference if more than small amounts of ultraviolet absorbing materials are present.

Wysokowski and Rewerski (51) reported determination of primidone in blood by measuring the absorbance of extracts at 225 nm, but they also suggested that metabolites might interfere.

6.32 Ultraviolet Determination as Phenobarbital

Bush and Helman (16) determined primidone in urine and blood as phenobarbital, after oxidation of the former with dichromate. Primidone and any phenobarbital in the samples were extracted with ether. The ether extracts were extracted with 0.1 M ethanolamine to remove phenobarbital, and then evaporated to dryness. The residue was treated with 0.042 M potassium dichromate in 9.5 M sulfuric acid to oxidize primidone to phenobarbital. The phenobarbital formed was extracted into ether, and the ether washed with pH 6.2 buffer, 0.1 N hydrochloric acid, and water. Finally, the phenobarbital formed was extracted into 0.1 M ethanolamine and determined from its ultraviolet absorption at pH 9.3, 7.3, and 5.3. Partition coefficients for primidone in several solvent systems were also determined.

6.33 Colorimetric Methods

Early assays for primidone in blood and tissue were done colorimetrically (52). The drug was extracted from the sample at pH 5.5 into a mixture of methyl acetate and chloroform. The extract was washed with pH 11.8 buffer and evaporated. The residue was treated by a method similar to that of Dill et al (50) for diphenylhydantoin. The metabolite phenylethylmalondiamide interferes, but can be removed after conversion with nitrous acid to α -phenyl-n-butyric acid, which is alkali-soluble (52).

Primidone can be hydrolyzed in 60 to 80 percent sulfuric acid, yielding formaldehyde (12,52) which can be determined with chromotropic acid. The

British Pharmacopoeia identification test for primidone is the appearance of a pinkish-blue color on heating with chromotropic acid solution (53).

6.4 Polarography

Bozsai and Vastagh (54) determined primidone by polarography after nitrating it with a sulfuric-nitric acid mixture. The phenyl group nitrates to form the 3'-nitrophenyl derivative (12), which is reducible at the dropping mercury electrode. The method was applied to tablets containing primidone, as well as to the raw material. The observed half-wave potential was -0.17 volt vs. the saturated calomel electrode.

6.5 Titration

Kalinowska et al (55) titrated primidone, in an acetone-water solution containing sodium hydroxide, with silver nitrate. The endpoint was a persistent turbidity, caused by precipitation of the di-silver salt of the drug (the mono-silver salt was soluble).

6.6 Other Analytical Tests

Barysheva (56) found that primidone showed a blue luminescence in the ultraviolet. Cooper (57) lists the colors formed by many drugs with p-dimethylamino-benzaldehyde; primidone forms a primrose color. Ammonia is evolved when primidone is heated with anhydrous sodium carbonate (17). Haug and Panescu (58) describe a rapid color test for detecting small amounts of phenobarbital or 5-phenyl-5-ethylthiobarbituric acid in primidone. Kassau (59), Kuhnert-Brandstätter et al (4), and Penprase and Biles (7) describe microscopic tests for primidone. Shamotienko (60) developed a color test for primidone, in which the same is boiled with chloramine, copper sulfate, and sodium hydroxide; primidone causes a reddish-violet color to develop. Lobanov and Gorbunova (61) determined primidone interferometrically in tablets and powders.

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PROPIOMAZINE HYDROCHLORIDE

Kathleen B. Crombie and Leo F. Cullen

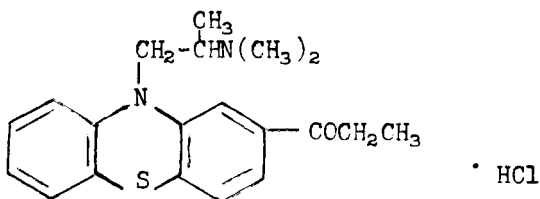
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1. Description

1.1 Name, Formula, Molecular Weight

Propiomazine hydrochloride is designated by the following chemical names: 10-(2-dimethylaminopropyl)-2-propionylphenothiazine hydrochloride¹, and propionyl-promethazine hydrochloride¹. In the subject indices of Chemical Abstracts, the compound is listed under the heading: 1-[10-[2-(dimethylamino) propyl] phenothiazin-2-yl]-1-propanone monohydrochloride. The most commonly used trade name is Largon Hydrochloride. The empirical formula is $C_{20}H_{24}N_2OS \cdot HCl$ with a molecular weight of 376.95.



1.2 Appearance, Color, Odor

Propiomazine hydrochloride is a yellow, practically odorless powder².

2. Physical Properties

2.1 Infrared Spectra

The infrared spectrum of propiomazine hydrochloride (N.F. Reference Standard material), presented in Figure 1, has been run in a KBr pellet. The following absorption band assignments have been made.³

Wavelength of Absorption (cm. ⁻¹)	Vibration Mode
2920	CH stretching
2580 and 2450	NH ⁺ stretching
1690	C=O stretching (aryl ketone)
1590	aromatic C=C in-plane vibration
	continued

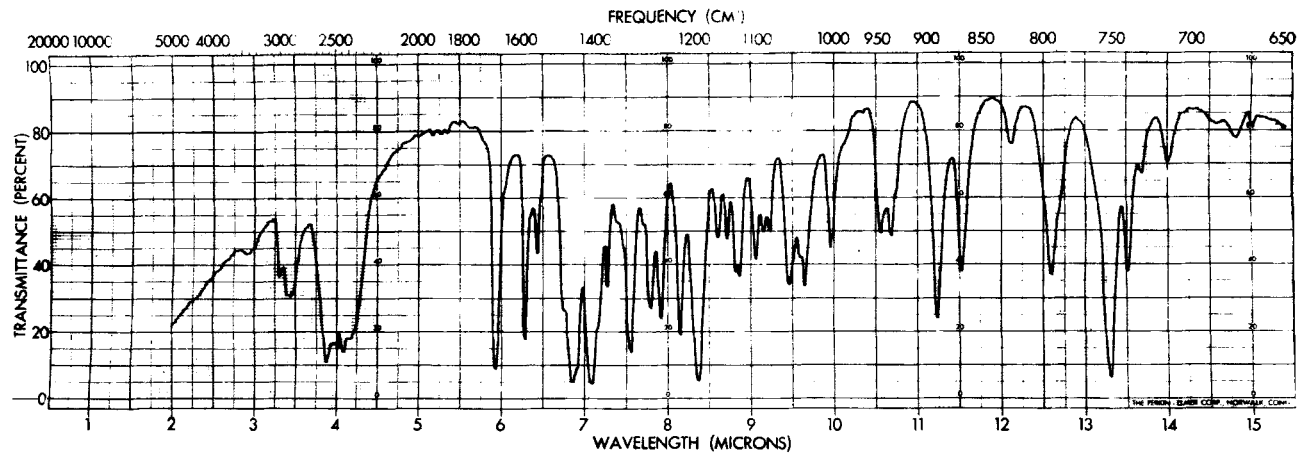


Figure 1. I.R. Spectrum of Propiomazine Hydrochloride (N.F. Reference Standard Material) - 1% KBr
Instrument: Perkin Elmer Model 21

2.1 Infrared Spectra (continued)

<u>Wavelength of Absorption (cm.⁻¹)</u>	<u>Vibration Mode</u>
1460	$\left\{ \begin{array}{l} \text{C-CH}_3 \text{ deformation} \\ \text{-CH}_2\text{- deformation} \\ \text{N-CH}_3 \text{ deformation} \\ \text{aromatic C-N stretching} \end{array} \right.$
1325	
1055	
890 and 867	
752	$\left\{ \begin{array}{l} \text{C-S stretching} \\ \text{aromatic CH out-of-plane deformation} \\ \text{(1,2,4-trisubstituted benzene ring)} \\ \text{aromatic CH out-of-plane deformation} \\ \text{(ortho-disubstituted benzene ring)} \end{array} \right.$

2.2 Nuclear Magnetic Resonance Spectra

The nuclear magnetic resonance (NMR) spectrum (Figure 2) was obtained by preparing a saturated solution of propiomazine hydrochloride (N.F. Reference Standard material) in deuteriochloroform containing tetramethylsilane as the internal standard. The only exchangeable proton is the hydrogen associated with HCl. The following NMR proton spectral assignments have been made.⁴

<u>Chemical Shift (ppm.)</u>	<u>Proton</u>	
1.20	$\begin{array}{c} \text{O} \\ \parallel \\ \text{C} - \text{CH}_2 - \text{CH}_3 \end{array}$	triplet
1.57	$\text{CH} - \text{CH}_2$	doublet
2.91	$\text{N} - (\text{CH}_3)_2$	singlet
3.10	$\begin{array}{c} \text{O} \\ \parallel \\ \text{C} - \text{CH}_2 - \text{CH}_3 \end{array}$	quartet
3.50 to 4.40	$\text{N} - \text{CH}_2 - \text{CH}$	---
4.97	$\text{N} - \text{CH}_2 - \text{CH}$	---
7.42 and 7.75	aromatic	---

2.3 Ultraviolet Spectra

Tompsett reported λ_{max} . at 242 $m\mu$ and 273 $m\mu$ for propiomazine hydrochloride in 1N hydrochloric acid.⁵ Propiomazine hydrochloride (N.F. Reference Standard material) when scanned between 350 $m\mu$ and 220 $m\mu$ exhibited λ_{max} . at 242 $m\mu$ (ϵ -67.5) and 273 $m\mu$ (ϵ -45.2).⁶ This spectrum is shown in Figure 3.

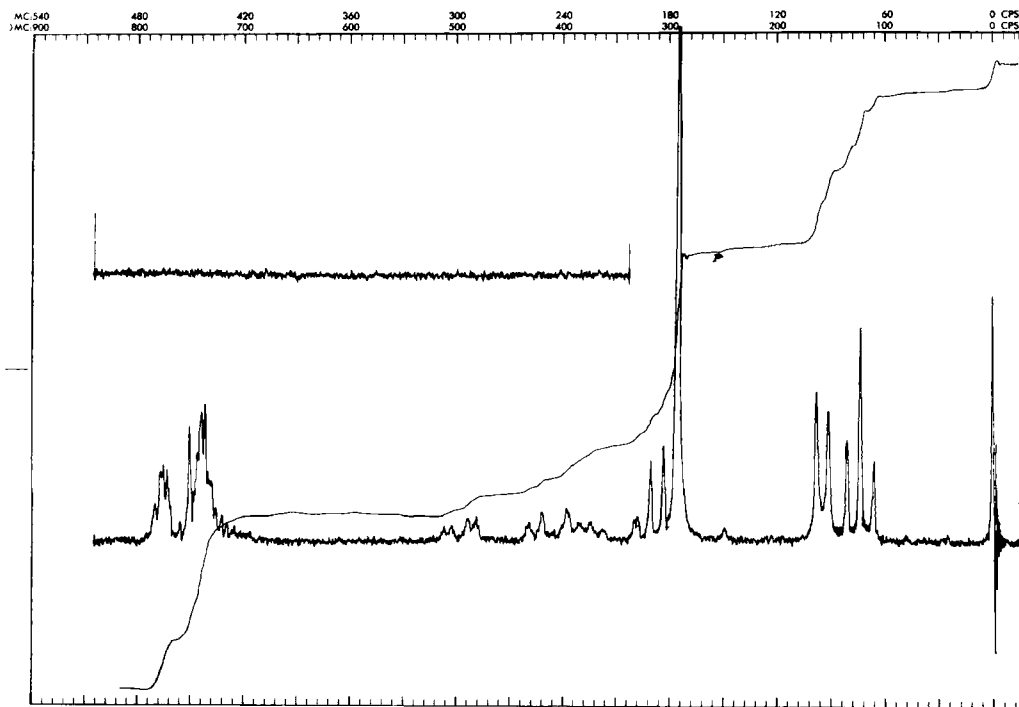


Figure 2. NMR Spectrum of Propiomazine Hydrochloride (N.F. Reference Standard Material) Solvent: deuteriochloroform, internal standard: tetramethylsilane
Instrument: Jeolco Model C-60 HL

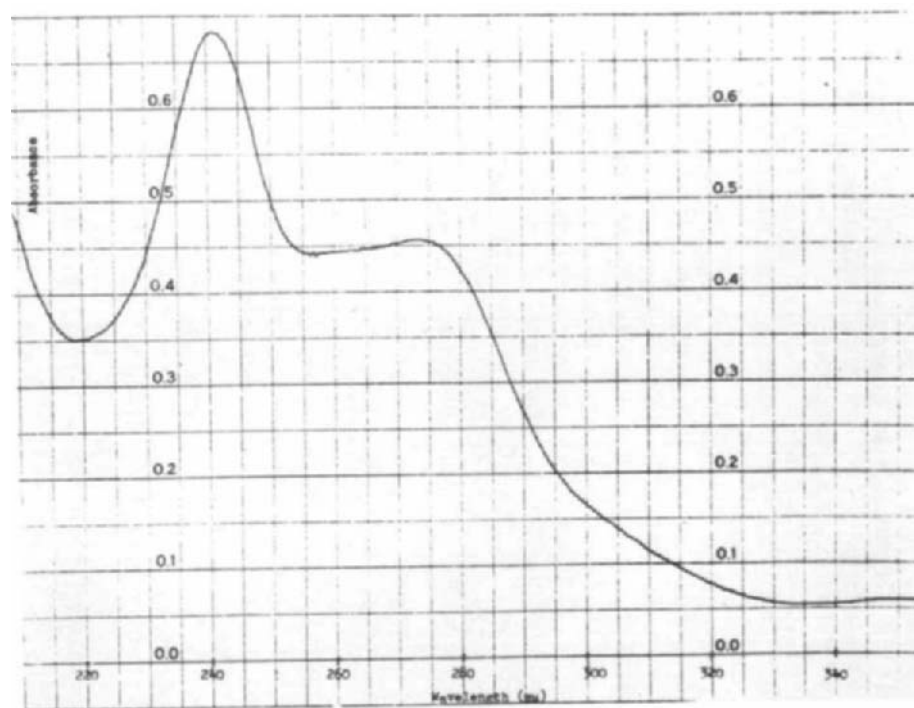


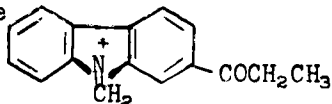
Figure 3. UV Spectrum of Propiomazine Hydrochloride (N. F. Reference Standard Material) Solvent: 1N hydrochloric acid
Instrument: Cary Model 14

2.4 Mass Spectrum

The mass spectrum of propiomazine hydrochloride (N.F. Reference Standard material) was obtained by direct insertion of the sample into an MS-902 double focusing mass spectrometer. The ion source temperature was 200°C. and the ionizing electron beam energy was 60 eV. The high resolution data was compiled and tabulated with the aid of an on-line PDP-8 Digital Computer.

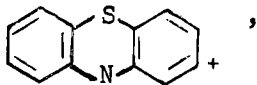
Results are presented as a bar graph (Figure 4) and the high resolution mass spectrum assignments of the prominent ions are given in Table I.

Propiomazine hydrochloride gives a molecular ion of the free base at m/e 340.1622. The first prominent fragment is at mass 269.0854 which corresponds to the loss of the metastable ion, C_1H_6N , supported by the fragment at 71.0736. This metastable ion yields a protonated fragment at mass 72.0806 which is the most abundant peak in the spectrum. Documented fission from the molecular ion of the side-chain C-C bond to the phenothiazine ring nitrogen generates the metastable 10-methylene-phenothiazinyl ion at m/e 268.0807 which loses the sulfur group to form the



ion of m/e 236.1107⁸. A peak at 254.0627 is indicative of the charged ion 2-propionyl-phenothiazin-10-yl resulting from the cleavage of the entire side chain attached to the 10-position ring nitrogen.

In the partial fragmentation of the 2-substituent, an ion of mass 225.0261 with the formula $C_{13}H_7ONS$ is observed which corresponds to the loss of C_2H_5 from the fragment at 254.0627. Cleavage of the entire 2-substituent from 2-propionyl-phenothiazin-10-yl yields a charged ion,



at m/e 197.0284.

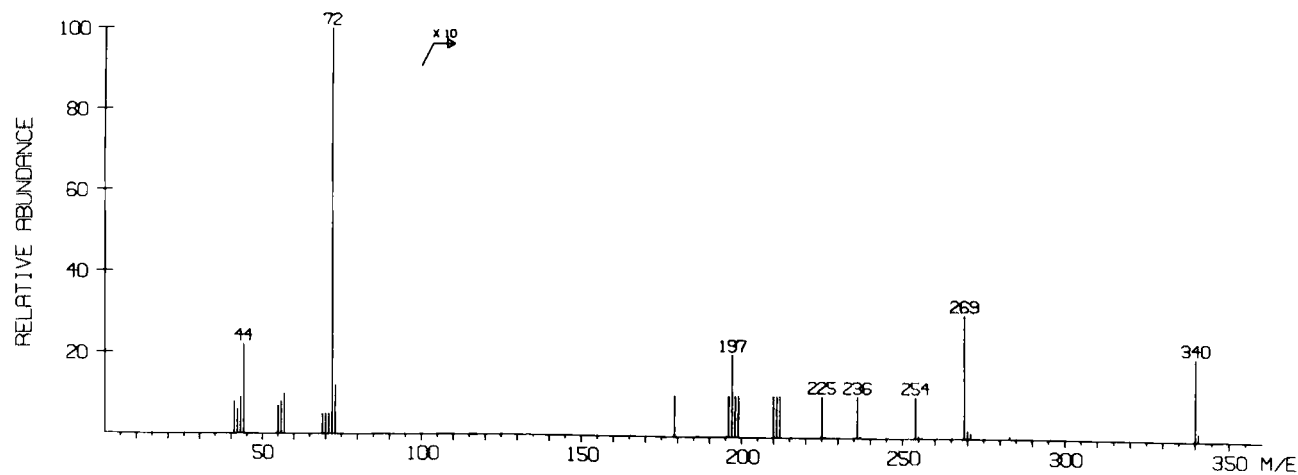


Figure 4. Mass Spectrum of Propiomazine Hydrochloride (N.F. Reference Standard Material)
Instrument: AEI-Model MS 902

TABLE IHigh Resolution Mass Spectrum Assignments
of Propiomazine HCl

<u>Measured Mass</u>	<u>Calculated Mass</u>	<u>Formula</u>
340.1622	340.1608	$C_{20}H_{24}ON_2S$
281.0891	281.0873	$C_{17}H_{15}ONS$
269.0854	269.0874	$C_{16}H_{15}ONS$
268.0807	268.0796	$C_{16}H_{14}ONS$
254.0627	254.0639	$C_{15}H_{12}ONS$
236.1107	236.1109	$C_{16}H_{14}ON$
225.0261	225.0247	$C_{13}H_7ONS$
197.0284	197.0298	$C_{12}H_7NS$
72.0806	72.0813	$C_4H_{10}N$
71.0736	71.0734	C_4H_9N

2.5 Melting Range

The following melting point temperatures ($^{\circ}\text{C}.$) have been obtained on propiomazine hydrochloride (N.F. Reference Standard material) employing the U.S.P. XVIII, Class 1, conditions:⁹ 201-204 with decomposition¹⁰.

2.6 Differential Thermal Analysis

The differential thermal analysis (DTA) curve of propiomazine hydrochloride (N.F. Reference Standard material) run from room temperature to the melting point exhibits no endotherms or exotherms other than an endotherm at $199^{\circ} - 203^{\circ}\text{C}.$ associated with the melt.¹⁰ Since the compound melts with decomposition, this endotherm is not well defined. The DTA curve was run on a Dupont 900 DTA using a micro cell and a heating rate of $5^{\circ}\text{C}./\text{min}.$

2.7 Solubility

The following approximate solubility data have been determined for propiomazine hydrochloride at room temperature:^{6,11}

water: greater than 1 g./ml.

isotonic sodium chloride solution: greater than 1g./ml.

95% ethanol: 100 mg./ml.

chloroform: 400 mg./ml.

acetone: 10 mg./ml.

benzene: less than 0.1 mg./ml.

ether: less than 0.1 mg./ml.

2.8 Crystal Properties

The X-ray powder diffraction pattern of propiomazine hydrochloride (N.F. Reference Standard material) obtained with a Philips diffractometer using $\text{Cu K}\alpha$ radiation¹⁰ is shown in Figure 5. The calculated d-spacings¹⁰ for the diffraction pattern shown in Figure 5 are given in Table II.

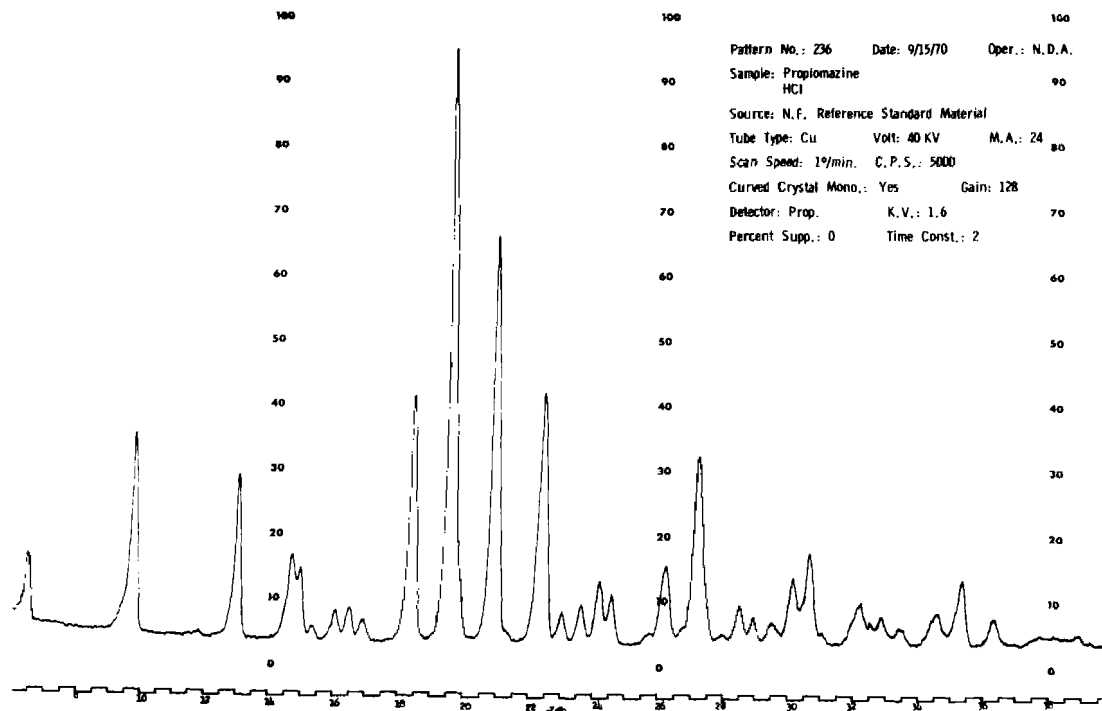


Figure 5. X-Ray Diffraction Pattern of Propiomazine Hydrochloride
 (N.F. Reference Standard Material) Radiation: Cu K α
 Instrument: Norelco Philips Diffractometer

PROPIOMAZINE HYDROCHLORIDE

TABLE II"d" Spacings for Propiomazine Hydrochloride

<u>2θ</u>	<u>d(Å)</u>
6.5°	13.60
9.8	9.03
13.1	6.76
14.7	6.02
15.0	5.91
18.5	4.79*
19.65	4.52*
21.0	4.23*
22.5	3.95*
23.05	3.86
23.7	3.75
24.25	3.67
24.6	3.62
26.3	3.39
27.25	3.27
28.5	3.13
29.0	3.08
30.2	2.96
30.7	2.91
32.25	2.78
34.6	2.59
35.8	2.51
36.3	2.47

*Most intense peaks

$$d = (\text{interplanar distance}) \frac{n\lambda}{2 \sin \theta}$$

2.9 Ionization Constant, pKa (Apparent)

The pKa for propiomazine hydrochloride has been determined potentiometrically to be 6.6 by aqueous titration with 0.1N sodium hydroxide.⁶

3. Synthesis

Two basic synthetic routes have been reported for the preparation of propiomazine hydrochloride. Farbenfabriken Bayer¹² prepared propiomazine by reacting 2-dimethylamino-propyl chloride with 2-propionylphenothiazine in the presence of sodium amide as the condensing agent, and subsequently converting the base form to the hydrochloride (See Figure 6).

An alternate synthesis was developed by Etablissements Clin-Byla¹³ which is based on the decarboxylation by heating of the aminoalkyl ester of the N-carboxy-phenothiazine. Specifically, 2-propionylphenothiazine is converted into its N-carbonyl chloride derivative by reacting with phosgene. Reaction of the chloride with 2-dimethylamino-1-propanol yields the ester hydrochloride, which is decarboxylated by heating (See Figure 7).

4. Stability - Degradation

Propiomazine hydrochloride is very stable in both the solid state¹⁴ and in buffered aqueous solutions exposed to long term accelerated thermal conditions which are protected from UV light and contain a suitable antioxidant system to exclude dissolved oxygen.¹⁵ It is common for the photocatalyzed oxidative decomposition of structurally related phenothiazine derivatives to proceed by a semi-quinone free radical mechanism.¹⁶⁻²⁰ J. Kofoed et.al.^{21,22} oxidized propiomazine to the corresponding 5-oxide or sulfoxide by reacting the compound with hydrogen peroxide at 60°C. Exposure of aqueous solutions of propiomazine hydrochloride to more severe thermal conditions has resulted in the cleavage of the compound to form 2-propionyl phenothiazine as an oxidative decomposition product.²³ A similar cleavage reaction was observed under accelerated thermal conditions for aqueous solutions of a somewhat structurally similar phenothiazine derivative, promethazine hydrochloride, resulting in the formation of 10-methylphenothiazine and phenothiazine.^{24,25}

PROPIOMAZINE HYDROCHLORIDE

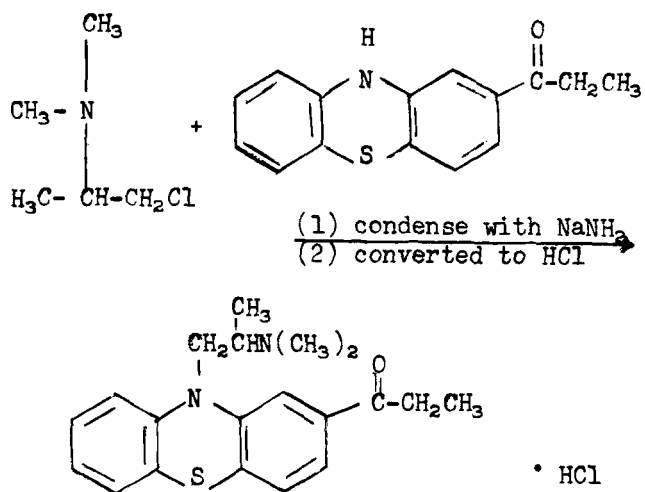


Fig. 6 -- Farbenfabriken Bayer Synthesis of Propiomazine HCl

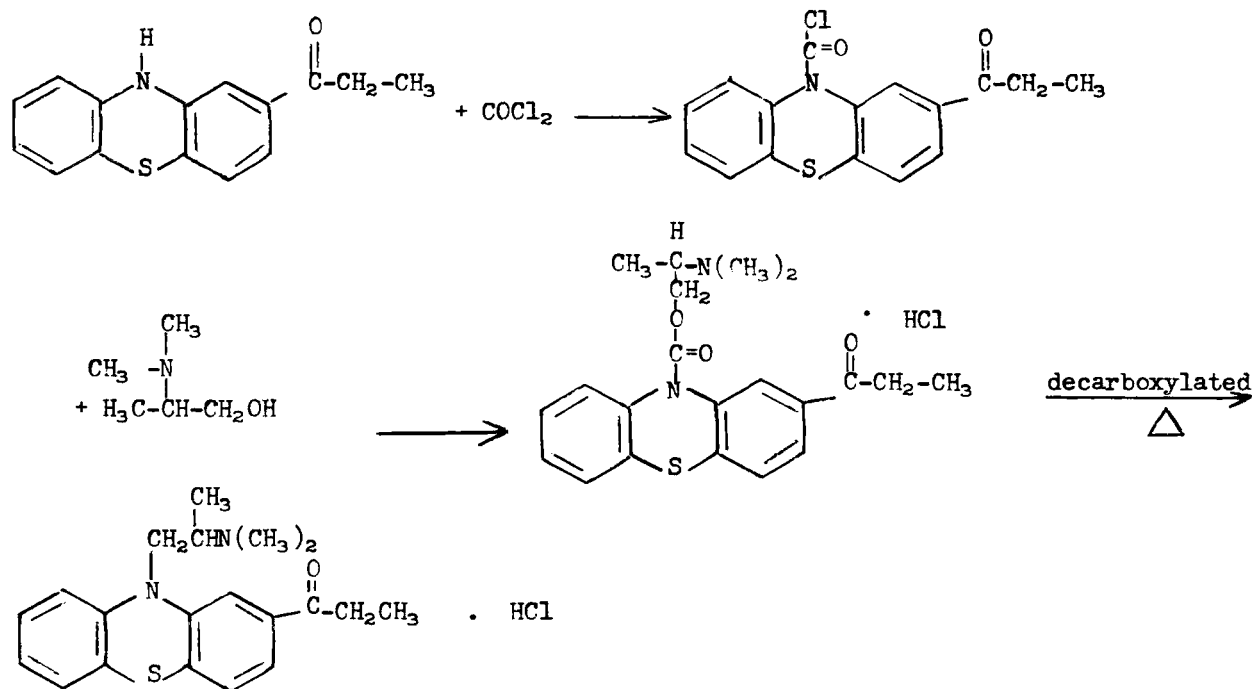


Fig. 7 -- Etablissements Clin-Byla Synthesis of Propiomazine HCl

5. Drug Metabolic Products

On incubation of propiomazine with a rat liver preparation, Robinson²⁶ characterized the predominant biotransformation product as the aromatic ring hydroxylated metabolite. The metabolic product resulting from dealkylation of the 10-dialkylaminoalkyl substituent, which is a characteristic metabolite of most biologically active phenothiazine derivatives, was not detected in this study.

6. Identification

Propiomazine hydrochloride can be identified by virtue of its characteristic IR, UV, and X-ray spectra (See 2.1, 2.3, and 2.8). The N.F. XIII¹ describes a test based on the formation of the maleate salt of propiomazine and measurement of the characteristic melting point (157° - 165°C.) of the maleate salt form. A series of microcrystalline derivative²⁷ and color identification tests²⁸ have been reported to detect and differentiate microgram quantities of propiomazine from other structurally related phenothiazine derivatives.

7. Methods of Analysis

7.1 Elemental Analysis*

<u>Element</u>	<u>% Theory</u>	<u>% Determined</u> ¹⁰
C	63.73	63.59
H	6.68	6.62
N	7.43	7.21
S	8.51	8.27
Cl	9.40	9.48

* Propiomazine HCl, N.F. Reference Standard material

7.2 Ultraviolet Spectrophotometric Analysis

The ultraviolet absorption maximum of propiomazine hydrochloride at about 240 mμ in aqueous solutions has been utilized for assay purposes¹. Determination of the absorptivity value is useful for assay of raw material and for formulations after the propiomazine has been isolated. Propiomazine hydrochloride could possibly be separated from interfering formulation components by an extraction of the base form with organic solvents from an alkaline aqueous solution.

Tompsett⁵ quantitatively determined propiomazine in biological material by comparing its absorbance to that of reference standard material in 1N hydrochloric acid after extracting the propiomazine from an alkaline aqueous phase with chloroform.

7.3 Colorimetric Analysis

7.31 Palladium Chloride

Modifications of the palladium-phenothiazine derivative complex procedure of Ryan¹⁸ have been applied to the quantitative analysis of propiomazine hydrochloride successfully.^{1,15} The colorimetric procedure is based on the reaction of palladium with propiomazine in an aqueous solution buffered at about pH 3 to form a colored complex which is spectrophotometrically measured at 465 mμ. Since this complex formation is based on an electron transfer from the sulfur moiety to the palladium ions, the procedure provides a method to assay propiomazine in the presence of its corresponding sulfoxide oxidative decomposition product.

7.32 p-Benzoquinone

A color reaction is obtained by the action of p-benzoquinone in a strongly acid medium on propiomazine²⁹. A stable colored species results which allows measurement of its absorption in the visible region. This redox reaction is based on the formation of the cationic radical of propiomazine with p-benzoquinone acting as an electron acceptor.

7.4 Polarographic Analysis

Propiomazine hydrochloride exhibits an anodic wave at +0.8v in acidic aqueous solution at a concentration range of 10^{-3} to 10^{-5} M using a stationary platinum electrode vs. a calomel reference electrode, suitable for a quantitative assay³⁰. The wave corresponds to the oxidation of the sulfur in the phenothiazine nucleus to the sulfoxide³¹.

7.5 Titrimetric Analysis

7.51 Sodium Lauryl Sulfate

Pellerin et.al.^{32,33} developed a semi-micro, heterogeneous phase (acidic aqueous-chloroform)

titrimetric procedure for propiomazine and related phenothiazine derivatives at the 0.05mM level employing 0.01M sodium lauryl sulfate as the titrant and methyl yellow as the indicator. The authors demonstrate that common tablet excipients do not interfere in the assay.

7.52 Ceric Sulfate

A spectrophotometric titration procedure employing 0.02N ceric sulfate as the titrant, used to assay a variety of phenothiazine derivatives and their dosage forms, can be applied to the quantitative analysis of propiomazine hydrochloride³⁴. Initially, a colored semi-quinone intermediate is formed which represents the first stage in the oxidation. Upon loss of a second electron, the solution becomes colorless as a result of the formation of the sulfoxide derivative of the phenothiazine. This mechanism for the oxidation of the phenothiazine under the conditions of the titration are described by Merkle and Discher³⁵. The endpoint is ascribed when excess ceric ion remains in solution and is characterized by an increase in absorbance, spectrophotometrically measured at 420 mμ, which is directly proportional to the ceric ion concentration. Employing a similar titrimetric procedure, Chatten, Locock and Krause³⁶ measured the changes in optical density of the reaction solution at 270 mμ, the wavelength of maximum absorbance for the intermediate semi-quinone free radical.

7.53 Perchloric Acid

The non-aqueous potentiometric titration of amine hydrochloride salts, introduced by Pifer-Wollish³⁷ and used in the assay of various phenothiazine derivatives⁹ can be applied to the determination of propiomazine hydrochloride. The procedure involves initial reaction of the amine hydrochloride group with mercuric acetate in an acetic acid medium. The halide is tied up as undissociated HgCl₂ and the acetate ion liberated can be titrated as a base with perchloric acid. Mercuric acetate is essentially undissociated in acetic acid and the excess, therefore, does not interfere.

7.6 Chromatographic Analysis

Chromatographic methods can be used qualitatively for identification and quantitatively for determination of purity and stability of propiomazine hydrochloride.

7.61 Thin Layer Chromatography

The various eluant and adsorbent systems used for thin layer chromatography of propiomazine hydrochloride are given in Table III. The visualization techniques used for the detection of propiomazine are also included in Table III.

7.62 Paper Chromatography

Many paper chromatographic methods have been reported in the literature to permit the isolation and detection of propiomazine hydrochloride. A summary of the available data is recorded in Table IV.

7.63 Gas Chromatography

Propiomazine hydrochloride has been chromatographed at an operating temperature of 225°C. on a 2.5% SE-30 Chromosorb W column and a 3% XE-60 silicone nitrile polymer on Chromosorb W column.²⁸ Employing a 3% SE-30 Diatoport S column operated at a temperature of 240°C., it is possible to separate propiomazine hydrochloride from its degradation product, 2-propionyl phenothiazine.⁶

Kofoed, et.al.,²² chromatographed propiomazine, as well as its sulfoxide, on a 3% SE-30 Gas Chrom Q column at temperatures of 210°C. and 250°C. Fontan, Jain, and Kirk³⁸ have developed an identification procedure for propiomazine and related phenothiazines based on examination of the characteristic gas chromatographic pattern of its pyrolysis products.

Table IIIThin Layer Chromatographic Systems for Propiomazine HCl

<u>Solvent System</u>	<u>Adsorbent</u>	<u>Visualization Technique</u>	<u>R_f</u>	<u>Reference</u>
A	Silica Gel G	A,B	0.52	39
B	Silica Gel G	A,B	0.66	39, 40
C	Silica Gel G	B	----	39
D	Silica Gel G with Fluorescent Indicator	C,D	0.77	5, 21
E	Silica Gel with 0.1N NaOH	E	0.42	40
F	Silica Gel with 0.1N NaOH	E	0.59	40, 41, 42
F	Silica Gel with 0.1N KHSO ₄	E	0.40	41, 42
G	Silica Gel with 0.1N NaOH	E	0.50	40, 41, 42
H	Silica Gel with 0.1N NaOH or 0.1N KHSO ₄	B,C, D,E	----	41
I	Silica Gel with 0.1N KHSO ₄	E	0.26	41, 42

continued....

Table III (continued)Thin Layer Chromatographic Systems for Propiomazine HCl

<u>Solvent System</u>	<u>Adsorbent</u>	<u>Visualization Technique</u>	<u>R_f</u>	<u>Reference</u>
J	Silica Gel G with Fluorescent Indicator	C	0.67	43
K	Silica Gel G	A,C,F, G,H,I, J	0.72	40
L	Silica Gel G with 0.1N NaOH	A,C,F, G,H,I, J	0.71	40, 44
M	Silica Gel G with Fluorescent Indicator	C	0.67	45
N	Silica Gel	C,K,L,M	0.92	26
O	Silica Gel	C	----	46
P	Silica Gel	C	----	46
Q	Silica Gel	C	----	46

Solvent Systems

A Cyclohexane:diethylamine (9:1)

B Methanol: NH₄OH (100:1.5)

C Chloroform:diethylamine (9:1)

D 0.4 M ammonium acetate in 80% methanol

E Cyclohexane:benzene:diethylamine (75:15:10)

continued....

Table III (continued)Thin Layer Chromatographic Systems for Propiomazine HClSolvent Systems (concluded)

- F Methanol
- G Acetone
- H Methyl acetate
- I 95% Ethanol
- J pyridine:methanol (50:50)
- K Benzene:ethanol: NH_4OH (95:15:5)
- L Chloroform:methanol (90:10)
- M Benzene:acetone: NH_4OH (37:7:1)
- N Chloroform:ethanol: NH_4OH (80:20:1)
- O Toluene:dimethylformamide (100:25)
- P Toluene:methanol:dimethylformamide (45:30:20)
- Q Acetone:methanol:triethanolamine (100:100:3)

Visualization Techniques

- A Acid Ferric Chloride [FeCl_3 in 20% HClO_4 :
50% HNO_3 (1:5:50)]
- B Potassium Iodoplatinate Reagent
- C Ultraviolet Light
- D Dragendorff Reagent

continued....

Table III (concluded)

Thin Layer Chromatographic Systems for Propiomazine HCl

Visualization Techniques (concluded)

- E 1% I₂ in methanol
- F Folin-Ciocalteu Reagent
- G Madelin Reagent
- H Cinnamaldehyde
- I Ehrlich Reagent [p-dimethylaminobenzaldehyde in
acetic acid - phosphoric acid]
- J Furfural
- K Sulfuric Acid Spray
- L Hydrobromic Acid
- M Sodium Metaperiodate

Table IVPaper Chromatographic Systems for Propiomazine HCl

<u>Solvent System</u>	<u>Paper</u>	<u>Paper Treatment</u>	<u>R_f</u>	<u>Visualization Technique</u>	<u>Reference</u>
A	Whatman No. 1	Buffered in 5% sodium dihydrogen citrate	0.77	A,B,C	28,47,48
B	Whatman No. 1 or No. 3	Impregnated with 10% tributyrin in acetone	0.07	A,B	28,49
C	Whatman No. 1 or No. 3	Impregnated with 10% tributyrin in acetone	----	A,B	28,50,51

Solvent SystemsVisualization Technique

A	0.02 M Citric Acid in 87% n-butanol	A	Ultraviolet Light
B	Acetate buffer (pH 4.6)	B	Potassium Iodoplatinate Reagent
C	Phosphate buffer (pH 7.4)	C	Bromocresol Green Spray

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Analytical Profile - Sulfamethoxazole

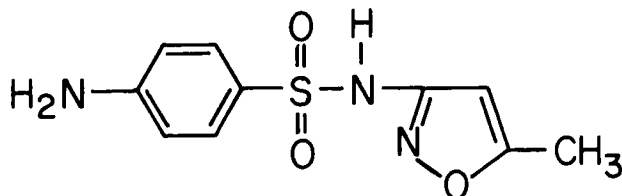
1. Description
 - 1.1 Name, Formula, Molecular Weight
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SULFAMETHOXAZOLE

1. Description

1.1 Name, Formula, Molecular Weight

Sulfamethoxazole is N¹-(5-methyl-3-isoxazolyl) sulfanilamide.



C₁₀H₁₁N₃O₃S

Molecular Weight: 253.28

1.2 Appearance, Color, Odor

Sulfamethoxazole occurs as a white to slightly off-white, practically odorless, crystalline powder.

2. Physical Properties

2.1 Infrared Spectrum

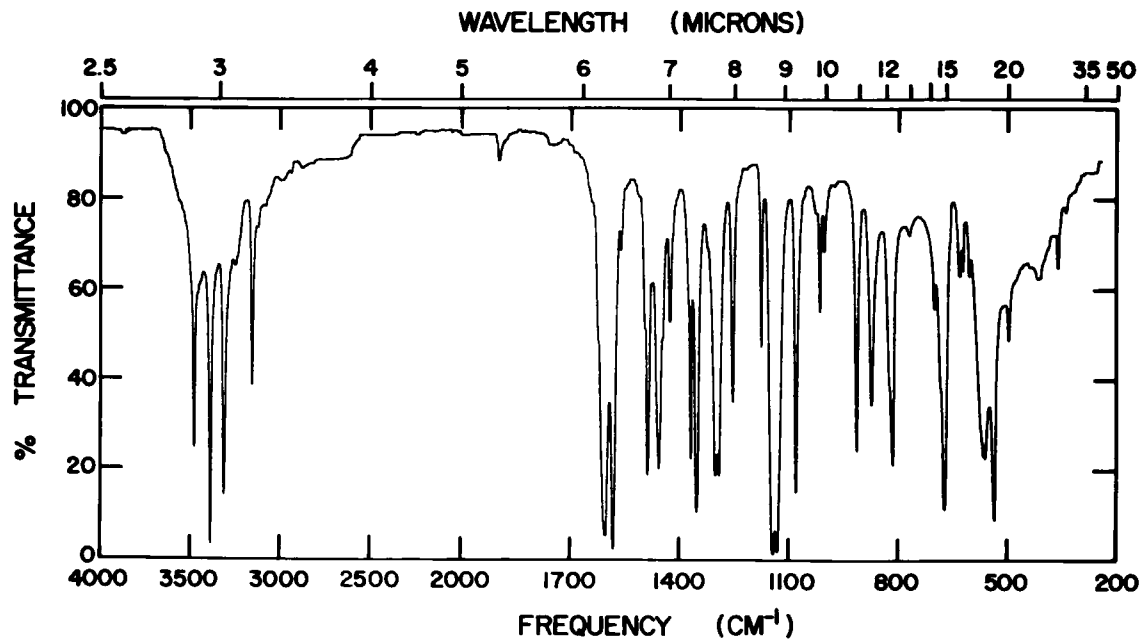
The infrared spectrum of reference standard sulfamethoxazole is presented in Figure 1 (1). The spectrum was measured with a Perkin-Elmer 621 Spectrophotometer in a KBr pellet containing 0.9 mg sulfamethoxazole/300 mg of KBr. Table I lists the assignments for the characteristic bands in the infrared spectrum (1).

TABLE I

Infrared Assignments for Sulfamethoxazole	
<u>Frequency (cm⁻¹)</u>	<u>Characteristic of</u>
3469 and 3379	NH ₂ stretch
3300	NH stretch
1616	Combination NH ₂ deformation and isoxazole ring stretch
1595 and 1500	Aromatic C=C stretch
1313 - 1303	Asymmetric SO ₂ stretch
1155 - 1143	Symmetric SO ₂ stretch
828	Two adjacent H's on benzene ring

Figure 1

Infrared Spectrum of Sulfamethoxazole



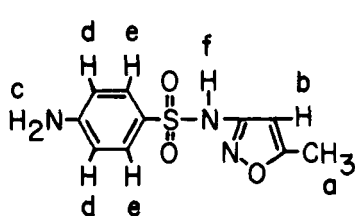
B. C. RUDY AND B. Z. SENKOWSKI

2.2 Nuclear Magnetic Resonance Spectrum

The spectrum shown in Figure 2 was obtained on a Jeol 60 MHz NMR by dissolving 53.6 mg of reference standard sulfamethoxazole in 0.5 ml of DMSO- d_6 containing tetramethylsilane as an internal reference (2). The spectral assignments are given in Table II (2). In order to establish the chemical shift for the single proton (H_b) on the isoxazole ring which appears as a shoulder on the broader primary amine protons peak, it was necessary to shift the amine protons peak to another place in the spectrum. This was accomplished by adding 8 μ l of DCl to form the quaternary salt. This caused the broad singlet to shift to 5.62 ppm and isolated a singlet at 6.10 ppm assignable to the single proton (H_b) on the isoxazole ring (Figure 2 - Insert).

TABLE II

NMR Assignments for Sulfamethoxazole

Structure	Protons	Chemical Shift	Multiplicity
		ppm	
	a	2.29	S
	b	6.10	S
	c	6.13	S(b)
	d	6.63	d($J_{H_d-H_e}=9\text{Hz}$)
	e	7.55	d($J_{H_e-H_d}=9\text{Hz}$)
	f	11.00	S(b)

S = sharp singlet; S(b) = broad singlet; d = doublet;
J = splitting constant in Hz

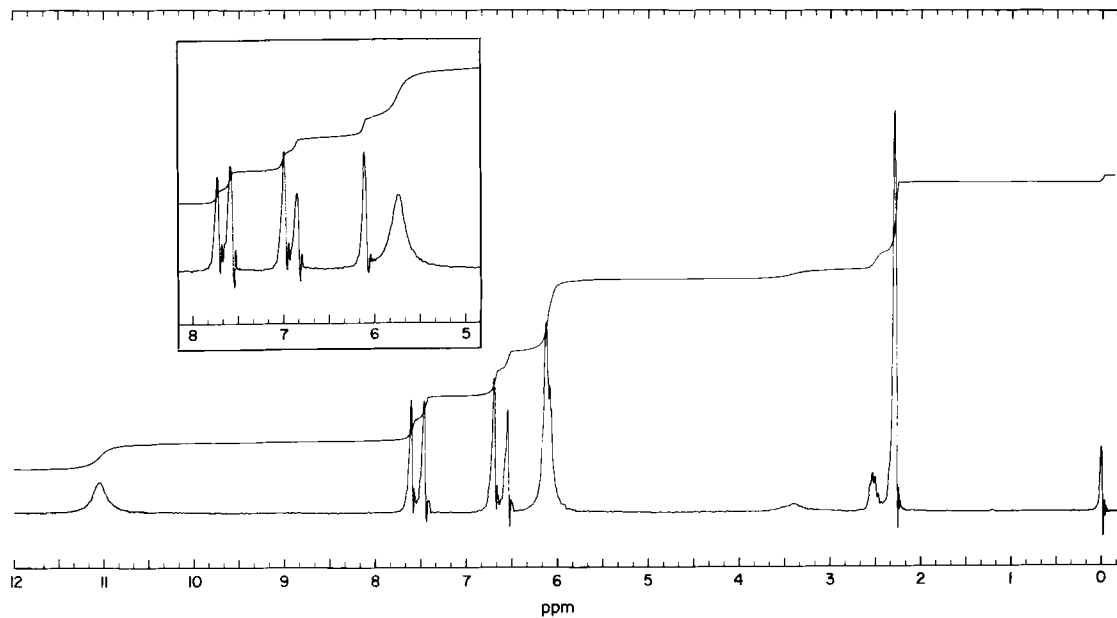
2.3 Ultraviolet Spectrum

When the UV spectrum of sulfamethoxazole was scanned between 350 to 215 nm, one maximum and one minimum were observed. The maximum is located at 256-257 nm ($\epsilon=1.72 \times 10^4$) and the minimum at 224-225 nm. The spectrum shown in Figure 3 was obtained from a solution of 1.025 mg sulfamethoxazole/100 ml of pH 7.5 phosphate buffer (3). This spectrum is the same as ones obtained using 0.1N NaOH as the solvent (4).

Figure 2

NMR Spectrum of Sulfamethoxazole

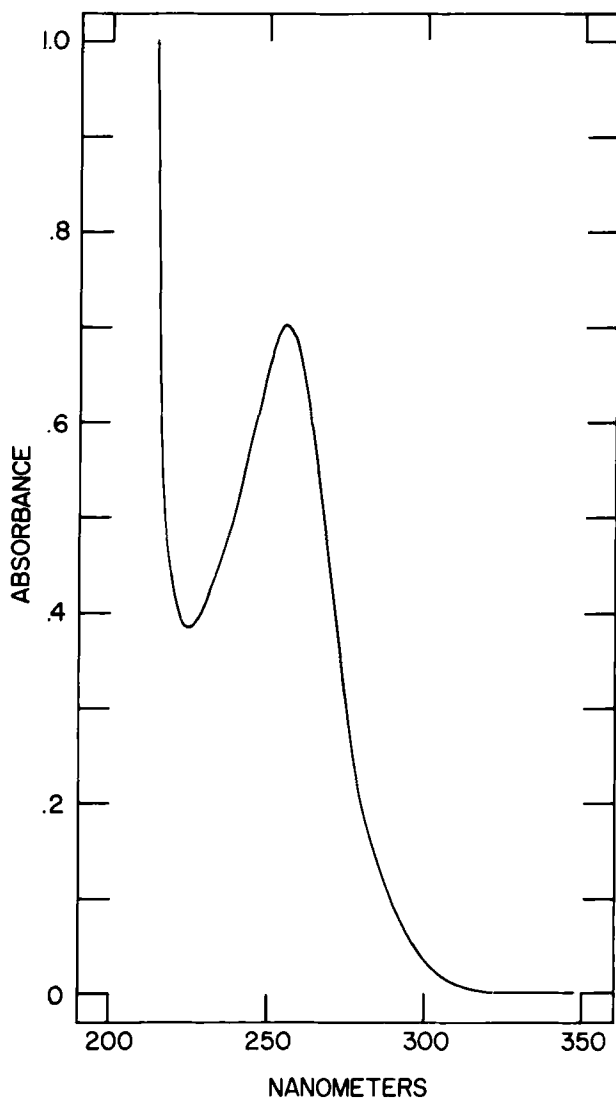
Insert: Spectrum in Region of 5 to 8 ppm
After Addition of DCl to Sulfamethoxazole



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Figure 3

UV Spectrum of Sulfamethoxazole



2.4 Fluorescence Spectrum

The excitation and emission spectra for sulfamethoxazole (1 mg/ml of methanol) are shown in Figure 4 (5). One maximum appears in the excitation spectrum at 314 nm and one maximum in the emission spectrum at 338 nm.

2.5 Mass Spectrum

The mass spectrum of sulfamethoxazole was obtained using a CEC 21-110 mass spectrometer with an ionizing energy of 70 ev. The output from the mass spectrometer was analyzed and presented in the form of a bar graph, shown in Figure 5, by a Varian 100 MS dedicated computer system (6). The peaks at m/e 92, 108, 140, and 156 correspond to $H_2N-C_6H_4$, $H_2N-C_6H_4-O$, $H_2N-C_6H_4-SO$, and $H_2N-C_6H_4-SO_2$, respectively. The peak m/e 189 is due to the loss of SO_2 from the parent and the peak at m/e 174 is due to the loss of both SO_2 and CH_3 from the parent. Loss of SO_2 is a typical rearrangement reaction with sulfonamides. The strong $M+2$ peak at 255 may be the ^{34}S isotope peak, however, this peak is not expected to be so intense (6).

2.6 Optical Rotation

Sulfamethoxazole exhibits no optical activity.

2.7 Melting Range

The melting range reported in NF XIII for sulfamethoxazole is 170° to 173° using the class I procedure (7).

2.8 Differential Scanning Calorimetry (DSC)

The DSC scan for sulfamethoxazole is shown in Figure 6. A melting endotherm is observed at 172° when the temperature program was $10^\circ/\text{minute}$. The ΔH_f was found to be 7.5 kcal/mole. The endotherm observed at 204°C is attributed to the decomposition of the sulfamethoxazole (8).

2.9 Thermogravimetric Analysis (TGA)

A thermal gravimetric analysis performed on sulfamethoxazole exhibited no loss of weight when it was heated to 105°C at a rate of $10^\circ/\text{min}$ (8).

2.10 Solubility

The solubility data for sulfamethoxazole obtained at 25°C are given in Table III (9).

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Figure 4

Fluorescence Spectrum of Sulfamethoxazole

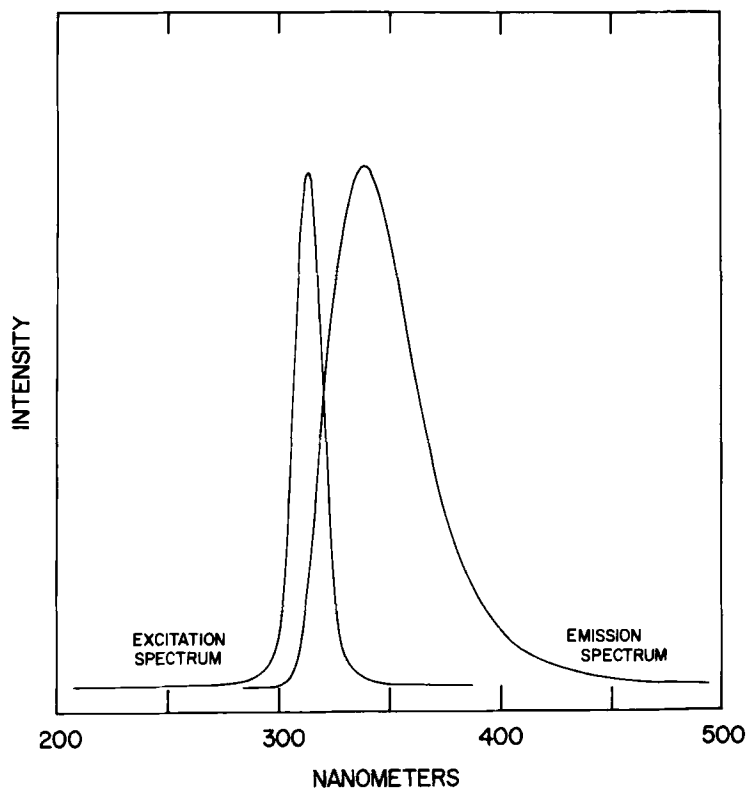
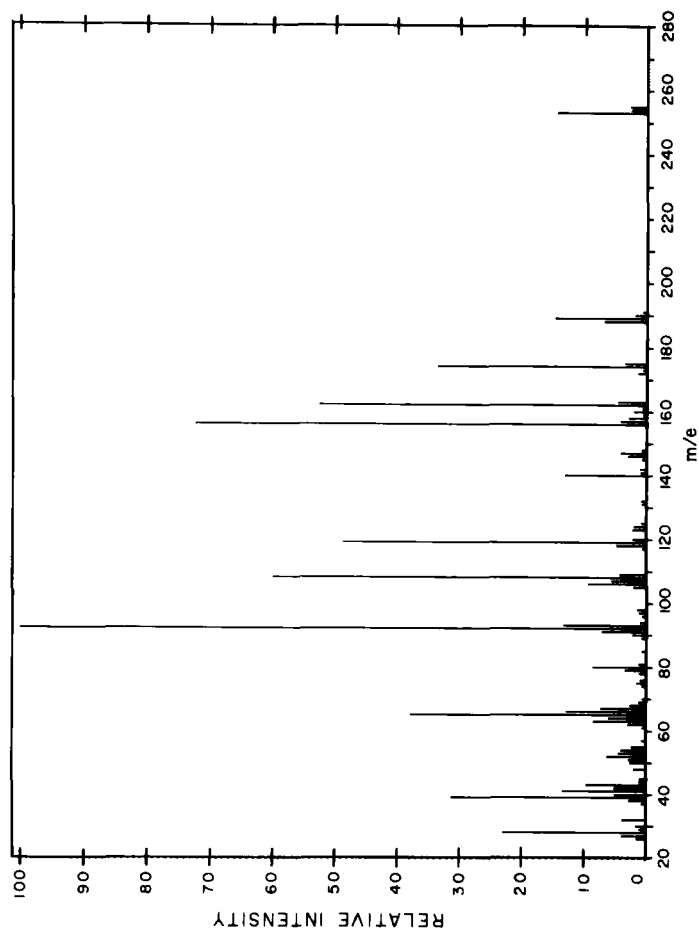


Figure 5
Mass Spectrum of Sulfamethoxazole



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Figure 6

DSC Curve for Sulfamethoxazole

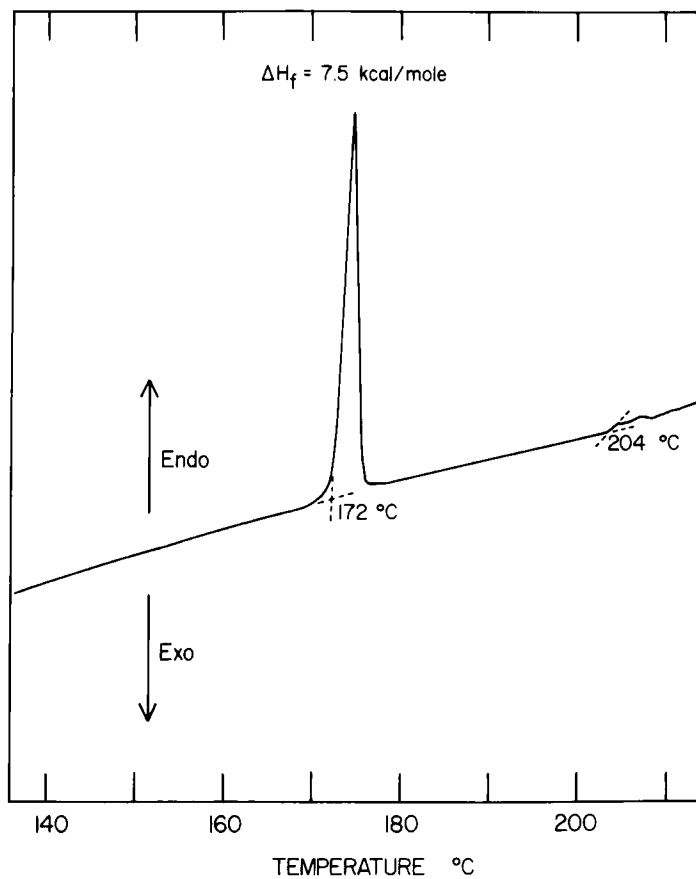


TABLE III

Solubility of Sulfamethoxazole in Different Solvents

<u>Solvent</u>	<u>Solubility (mg/ml)</u>
3A alcohol	30.6
benzene	0.5
chloroform	2.3
95% ethanol	37.8
ethyl ether	2.7
isopropanol	8.8
methanol	90.3
petroleum ether 30°-60°	0.2
0.1N NaOH	16.0
water	0.5

2.11 X-ray Crystal Properties

The x-ray powder diffraction pattern of sulfamethoxazole is presented in Table IV (10). The instrumental conditions are given below.

Instrument Conditions:

General Electric Model XRD-6 Spectrogoniometer
 Generator: 50KV-12-1/2 MA
 Tube target: Copper
 Radiation: Cu $K\alpha$ = 1.542 Å
 Optics: 0.1° Detector slit
 M.R. Soller slit
 3° Beam slit
 0.0007 inch Ni filter
 4° take off angle
 Goniometer: Scan at 0.2° 2 θ per minute
 Detector: Amplifier gain - 16 coarse,
 8.7 fine
 Sealed proportional counter tube
 and DC voltage at plateau
 Pulse height selection E_L -5 volts
 Eu - out, Rate meter T.C. 4
 2000 C/S full scale
 Recorder: Chart speed - 1 in./5 min.
 Samples: Prepared by grinding at room temp.

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TABLE IV

X-ray Powder Diffraction Pattern of Sulfamethoxazole

2θ	$d(\text{\AA})^*$	I/I_o^{**}	2θ	$d(\text{\AA})^*$	I/I_o^{**}
6.86	12.90	2	30.06	2.92	2
11.02	8.03	10	32.22	2.78	15
12.32	7.18	43	33.37	2.69	5
13.74	6.44	5	34.50	2.60	5
16.70	5.31	7	34.92	2.57	8
17.03	5.21	12	36.42	2.47	1
17.48	5.07	50	36.95	2.43	4
18.12	4.90	4	37.68	2.39	4
18.56	4.78	16	38.25	2.35	1
19.62	4.52	20	38.80	2.32	1
20.68	4.29	43	39.52	2.28	3
21.56	4.12	40	40.71	2.22	1
22.39	3.97	24	41.70	2.17	4
23.28	3.82	4	43.32	2.09	4
23.86	3.73	100	43.68	2.07	3
24.68	3.61	15	45.10	2.01	4
26.20	3.40	5	45.80	1.98	2
26.44	3.37	5	46.38	1.96	2
27.40	3.25	38	49.00	1.86	3
28.76	3.10	17	50.72	1.80	2
29.22	3.06	5			

* $d = (\text{interplanar distance}) \frac{n\lambda}{2 \sin \theta}$

** $I/I_o =$ relative intensity (based on highest intensity of 1.00)

2.12 Dissociation Constant

The pK_a for sulfamethoxazole has been determined spectrophotometrically to be 5.55 ± 0.05 and by titration of sulfamethoxazole in an excess of 0.1N HCl to be 5.63 ± 0.03 (11). These values agree well with the pK_a value of 5.60 at 25°C reported by Nakagaki, Toga, and Terada (12).

3. Synthesis

Sulfamethoxazole is prepared by the reaction scheme shown in Figure 7. 5-methyl-3-aminoisoxazole is reacted with N-acetyl-p-amino-benzene sulfonyl chloride. The acetyl group is then cleaved to yield sulfamethoxazole (11).

4. Stability Degradation

When 10% solutions of sulfamethoxazole in 0.4 N NaOH and in water were refluxed for one hour, no decomposition was observed by thin-layer chromatography (1). When sulfamethoxazole is refluxed in 0.4 N HCl, the molecule first cleaves to yield sulfanilic acid and 5-methyl-3-aminoisoxazole (1,11) and on extended heating in the HCl solution, 3 additional diazotizable products detectable by paper chromatography are formed (11). Pure sulfamethoxazole was found to be stable when subjected to a 110°C temperature for 5 days (11).

5. Drug Metabolic Products

Sulfamethoxazole is metabolized to its N₄-acetyl derivative which is the major form found in human urine (13,14). The intact drug along with three other metabolites which have not been completely identified are also present in lesser quantities in human urine (13). In human blood the sulfamethoxazole exists almost entirely as the intact drug (13).

6. Methods of Analysis

6.1 Elemental Analysis

The results from the elemental analysis are listed in Table V (15).

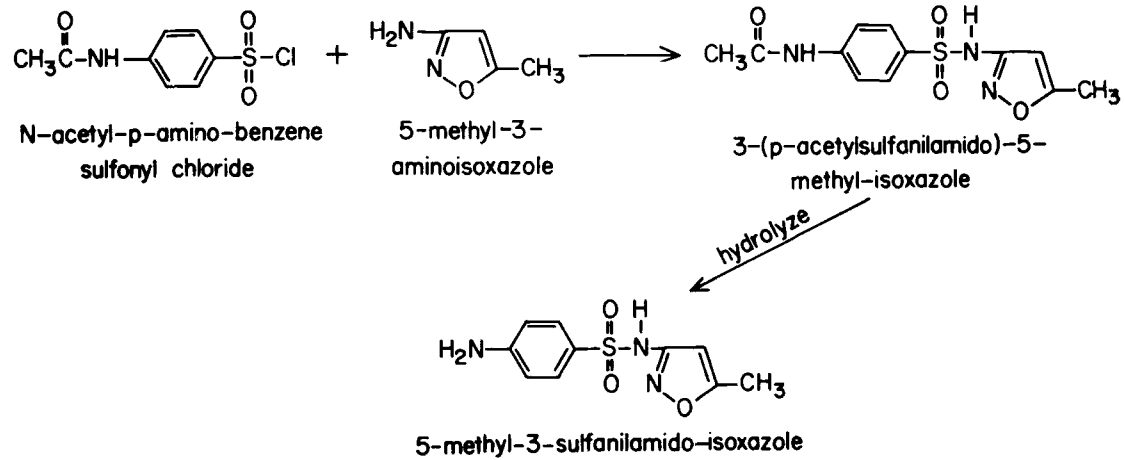
TABLE V

Elemental Analysis of Sulfamethoxazole

<u>Element</u>	<u>% Theory</u>	<u>% Found</u>
C	47.43	47.28
H	4.38	4.38
N	16.60	16.84
S	12.65	12.74

Figure 7

Synthesis of Sulfamethoxazole



6.2 Phase Solubility Analysis

Phase solubility analysis is carried out using isopropanol as the solvent. A typical example is shown in Figure 8 which also lists the conditions under which the analysis was carried out (9).

6.3 Thin Layer Chromatographic Analysis

The following TLC procedure is useful for separating sulfamethoxazole from sulfanilic acid and sulfanilamide (16). Using silica gel G plates and a solvent made from a mixture of alcohol, n-heptane, chloroform, and glacial acetic acid (25:25:25:7), spot 0.1 mg of sulfamethoxazole dissolved in ammoniacol methanol and subject to ascending chromatography. After development of at least 12 cm, the plate is air dried and sprayed with Modified Ehrlich's Reagent (100 mg p-dimethylamino-benzaldehyde in 1 ml concentrated hydrochloric acid diluted to 100 ml with 3 A alcohol). The approximate R_f values are:

Sulfanilic Acid	0.1
Sulfanilamide	0.5
Sulfamethoxazole	0.7

A solvent system which can be used to separate sulfamethoxazole from its N^4 -acetyl metabolite is chloroform:heptane:alcohol (1:1:1). Following the same basic procedure as above, the R_f value for sulfamethoxazole is 0.75 and for the N^4 -acetyl derivative 0.42 (3).

6.4 Direct Spectrophotometric Analysis

Direct spectrophotometric analysis of sulfamethoxazole may be carried out using 0.1N NaOH as the solvent. In this solvent Beer's Law holds between 0.26 $\mu\text{gm/ml}$ to 26 $\mu\text{gm/ml}$ (1×10^{-6} M to 1×10^{-4} M) (17).

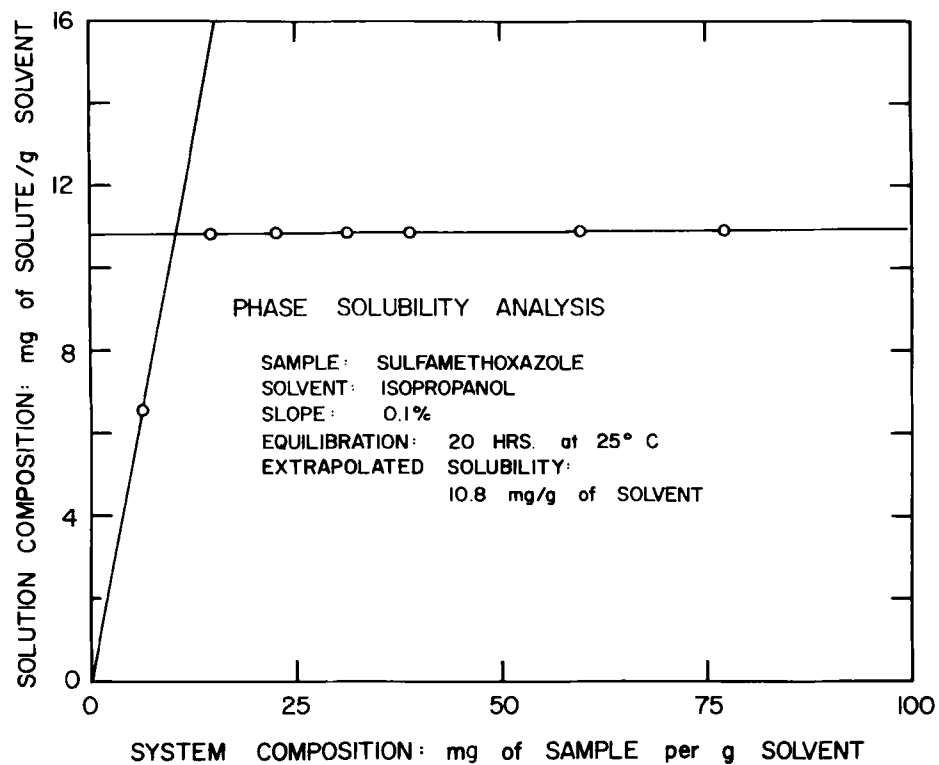
6.5 Colorimetric Analysis

The colorimetric analysis of sulfamethoxazole is accomplished by diazotization and coupling with N-(1-Naphthyl ethylenediamine dihydrochloride (Bratton-Marshall Reagent). The resultant red color is measured at 540 nm (18).

6.6 Fluorimetric Analysis

Fluorescence analysis has been used to determine the concentration of sulfamethoxazole in blood (19). For

Figure 8



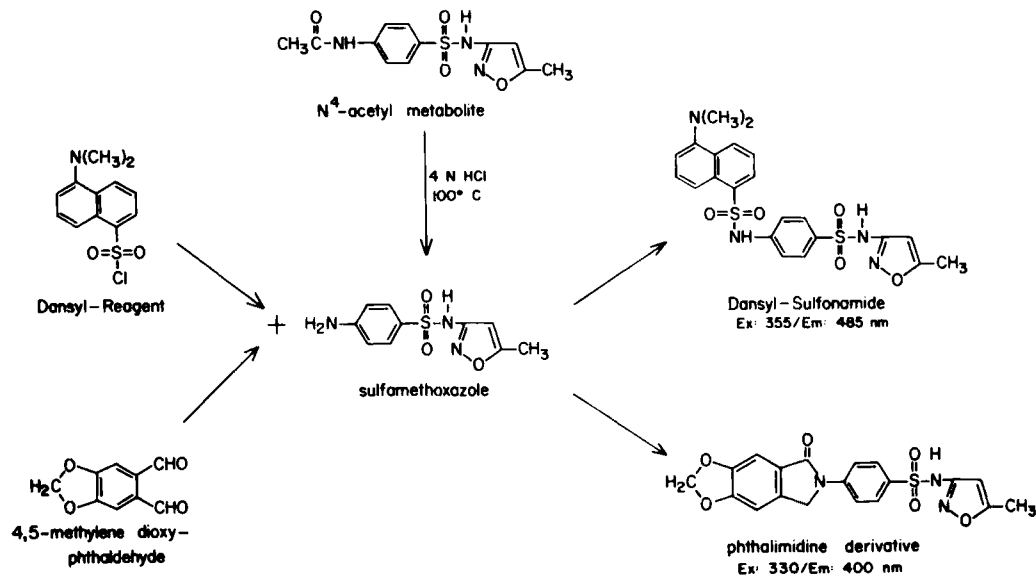
this purpose detectable levels in the range of 1 μg per ml of blood are needed. In order to achieve this sensitivity it is necessary to make a fluorescent derivative of sulfamethoxazole. One such derivative is the highly fluorescent "Dansylsulfonamide" which is formed by the reaction shown in Figure 9. The fluorescence yield of this compound is sufficient to measure about 1 μg of sulfamethoxazole per ml of blood. An even more sensitive derivative is formed when sulfamethoxazole is reacted with 4,5-methylene dioxy phthaldehyde (Figure 9). The sensitivity limit for this derivative is from 1×10^{-8} to 1×10^{-10} mole/ml (20).

6.7 Titrimetric Analysis

The potentiometric titration with sodium nitrite is the method of choice to assay sulfamethoxazole (16). The dried sample is dissolved in glacial acetic acid and water (1:2). Hydrochloric acid is added, the solution cooled to 15°C , and immediately titrated with 0.1M sodium nitrite. The end point is determined potentiometrically using calomel and platinum electrodes. Each ml of 0.1M sodium nitrite is equivalent to 25.33 mg of sulfamethoxazole.

Sulfamethoxazole can also be titrated in dimethylformamide with 0.1N LiOCH_3 using thymol blue as the indicator (17). The proton on the sulfonamide function is replaced by the lithium ion. Each ml of 0.1N lithium methoxide is equivalent to 25.33 mg of sulfamethoxazole.

Figure 9
Fluorescence Derivatives of Sulfamethoxazole



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Analytical Profile - Sulfisoxazole

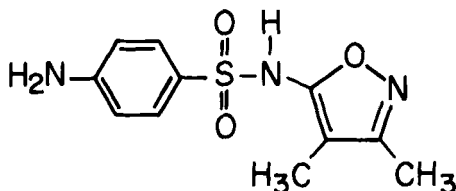
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 - 6.6 Fluorimetric Analysis
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SULFISOXAZOLE

1. Description

1.1 Name, Formula, Molecular Weight

Sulfisoxazole is N¹-(3,4-dimethyl-5-isoxazolyl) sulfanilamide.



C₁₁H₁₃N₃O₃S

Molecular Weight: 267.31

1.2 Appearance, Color, Odor

Sulfisoxazole occurs as a white to slightly yellowish, odorless, crystalline powder.

2. Physical Properties

2.1 Infrared Spectrum

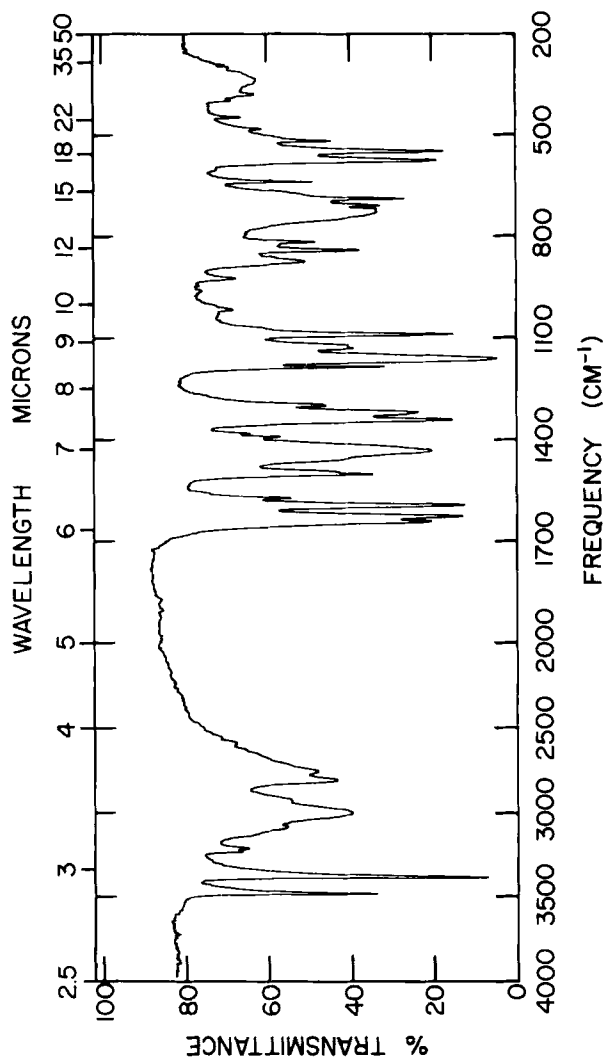
The infrared spectrum of sulfisoxazole is presented in Figure 1 (1). The spectrum was measured with a Perkin-Elmer 621 Spectrophotometer in a KBr pellet containing 1.0 mg sulfisoxazole/300 mg of KBr. Table I lists the assignments for the characteristic bands in the infrared spectrum (1).

Table I

Infrared Assignments for Sulfisoxazole

<u>Frequency (cm⁻¹)</u>	<u>Characteristic of</u>
3485 and 3380	Asymmetric and symmetric NH stretch
1629	NH ₂ deformation vibrations
1595 and 1503	Aromatic C=C stretch
1343 and 1160	Asymmetric and symmetric SO ₂ stretch
840	Two adjacent H's on phenyl ring

Figure 1
Infrared Spectrum of Sulfisoxazole



2.2 Nuclear Magnetic Resonance Spectrum (NMR)

The spectrum shown in Figure 2 was obtained on a Jeolco 60 MHz NMR by dissolving 60.9 mg of sulfisoxazole in 0.5 ml of DMSO-d₆ containing tetramethylsilane as an internal reference. The spectral assignments are given in Table II (2).

Table II

NMR Assignments for Sulfisoxazole

<u>Type of Protons</u>	<u>Chemical Shift ppm</u>	<u>Multiplicity</u>
CH ₃ on no. 4 isoxazole carbon	1.63	Sharp singlet
CH ₃ on no. 3 isoxazole carbon	2.05	Sharp singlet
NH ₂ on benzene ring	~6.1	Broad singlet
Aromatic protons ortho to amine moiety	6.58	Doublet (J=9Hz)
Aromatic protons ortho to sulfonamide moiety	7.42	Doublet (J=9Hz)
NH adjacent to isoxazole ring	~10.5	Broad singlet

2.3 Ultraviolet Spectrum (UV)

When the UV spectrum of sulfisoxazole was scanned from 350 to 205 nm, one maximum and one minimum were observed. The maximum is located at 253 nm ($\epsilon = 2.1 \times 10^4$) and the minimum at 222 nm. The spectrum shown in Figure 3 was obtained from a solution of 0.9998 mg sulfisoxazole/100 ml of pH 7.5 phosphate buffer (3).

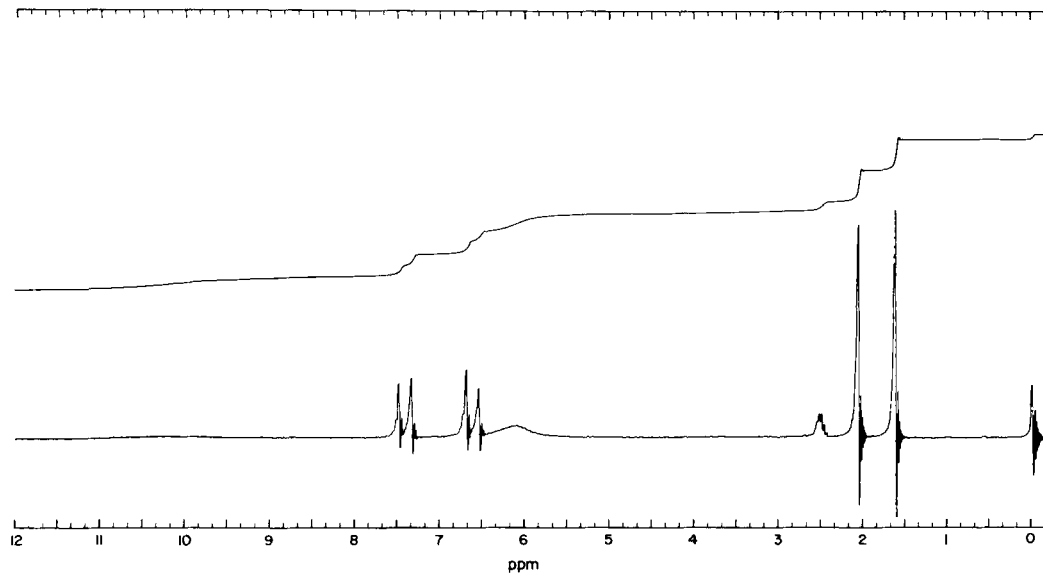
2.4 Fluorescence Spectrum

The excitation and emission spectra for sulfisoxazole (1 mg/ml of methanol) are shown in Figure 4 (4). One maximum appears in the excitation spectrum at 319 nm and one maximum in the emission spectrum at 336 nm.

2.5 Mass Spectrum

The mass spectral data for sulfisoxazole was obtained using a CEC 21-110 mass spectrometer with an ionizing energy of 70 ev. An on-line computer, Varian Data Systems 100 MS, was used to calculate the masses and compare the intensities to the base peak. The mass spectrum obtained from this computer analysis is shown in Figure 5

Figure 2
NMR Spectrum of Sulfisoxazole



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Figure 3

UV Spectrum of Sulfisoxazole

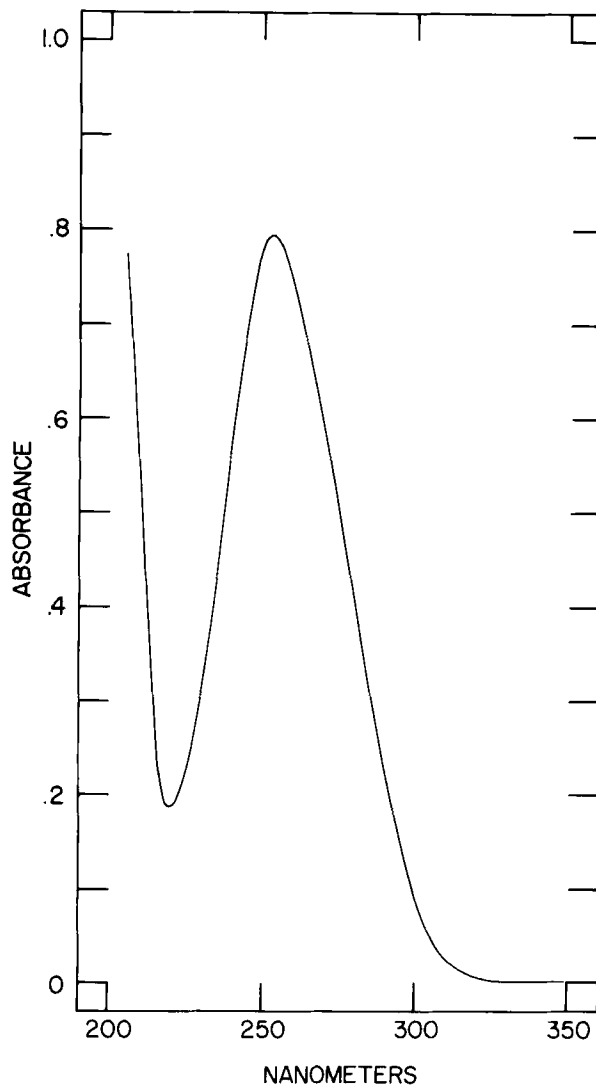


Figure 4
Fluorescence Spectrum of Sulfisoxazole

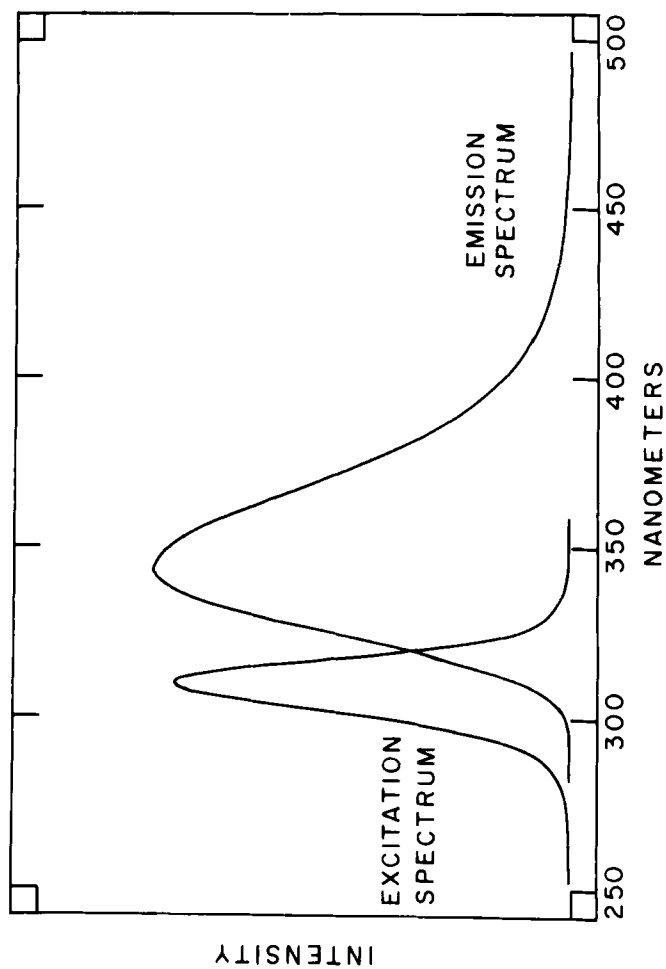
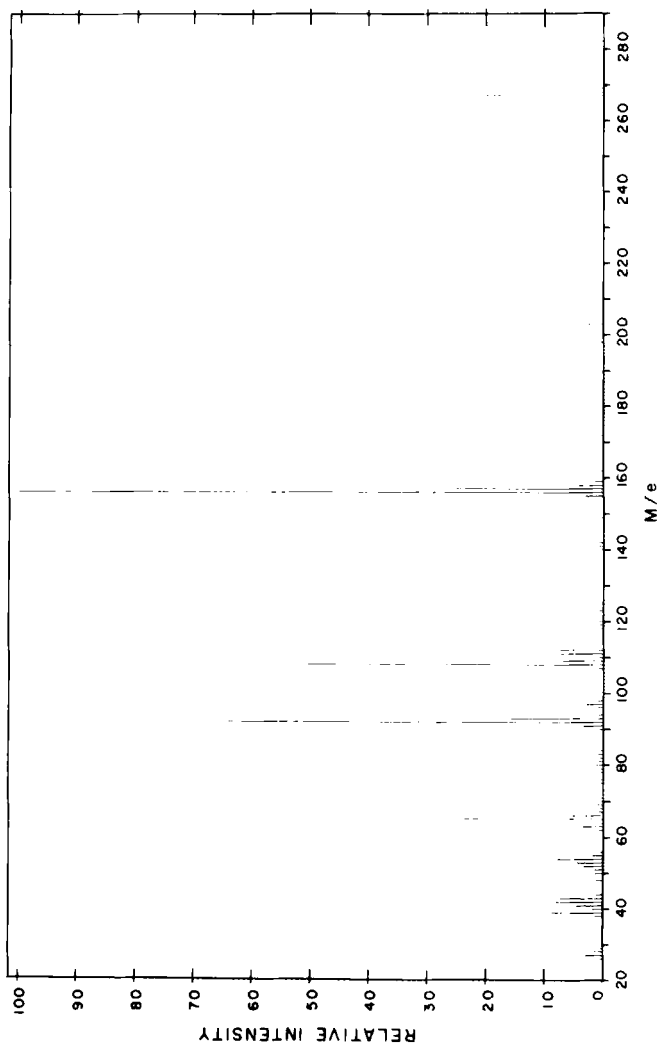


Figure 5
Mass Spectrum of Sulfisoxazole



(5). The peaks at m/e 92, 108, 140, 156, and 172 correspond to $H_2N-C_6H_4$, $H_2N-C_6H_4-O$, $H_2N-C_6H_4-SO$, $H_2N-C_6H_4-SO_2$, and $H_2N-C_6H_4-SO_2-NH_2$, respectively. The peak m/e 203 is due to the loss of SO_2 from the parent and the peak at m/e 198 is due to the loss of C_4H_7N from the parent. The loss of SO_2 is a typical rearrangement reaction with sulfonamides (5).

2.6 Optical Rotation

Sulfisoxazole exhibits no optical activity.

2.7 Melting Range

The melting range reported in USP XVIII for sulfisoxazole is 194° to $199^\circ C$ using the class Ia procedure (6).

2.8 Differential Scanning Calorimetry (DSC)

The thermal properties of sulfisoxazole in the melting region depend greatly on the previous thermal history of the sample. When a DSC scan for sulfisoxazole was obtained using a heating rate of $10^\circ C/minute$, a melting endotherm starting at $191.6^\circ C$ and a decomposition exotherm starting at $201.7^\circ C$ were observed (Figure 6). Because of the sample instability in the melting region, the ΔH_f was not obtained (7).

2.9 Thermogravimetric Analysis (TGA)

No weight loss was observed by TGA between ambient and $225^\circ C$ when the sample was heated at $10^\circ C/minute$. A weight loss began about $225^\circ C$ and amounted to about 60% of the sample weight at $475^\circ C$ (7).

2.10 Solubility

The solubility data for sulfisoxazole obtained at $25^\circ C$ are given in Table III (8).

Table III

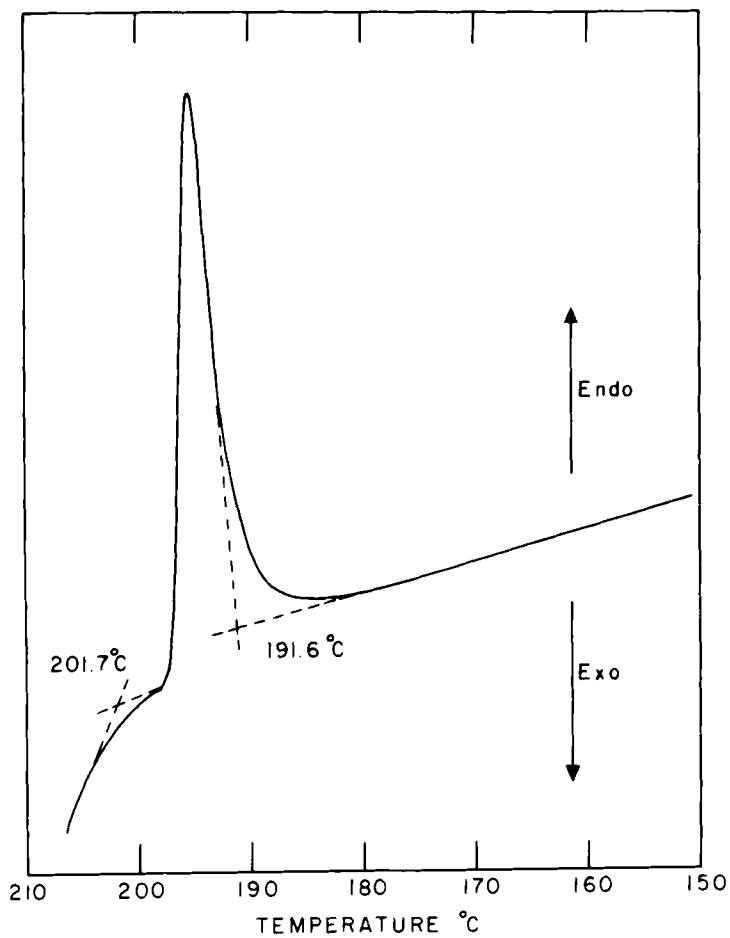
Solubility of Sulfisoxazole in Different Solvents

<u>Solvent</u>	<u>Solubility (mg/ml)</u>
3A alcohol	18.6
benzene	2.1

SULFISOXAZOLE

Figure 6

DSC Curve for Sulfisoxazole



chloroform	2.8
95% ethanol	10.5
ethyl ether	2.5
methanol	14.9
petroleum ether 30°-60°	1.4
2-propanol	8.6
water	0.2

2.11 X-ray Crystal Properties

The x-ray powder diffraction pattern of sulfis-oxazole is presented in Table IV (9). The instrumental conditions are given below.

Instrument Conditions:

General Electric Model XRD-6 Spectrogoniometer

Generator:	50KV-12-1/2 MA
Tube target:	Copper
Radiation:	Cu K_{α} = 1.542 Å
Optics:	0.1° Detector slit
	M.R. Soller slit
	3° Beam slit
	0.0007 inch Ni filter
	4° take off angle
Goniometer:	Scan at 0.2° 2θ per minute
Detector:	Amplifier gain - 16 course,
	8.7 fine
	Sealed proportional counter
	tube and DC voltage at
	plateau
	Pulse height selection E_L -5
	volts; Eu - out
	Rate meter T.C. 4
	2000 C/S full scale
Recorder:	Chart speed ~ 1 inch per 5
	minutes

Samples prepared by grinding at room temperature.

SULFISOXAZOLE

Table IV

X-ray Powder Diffraction Pattern of Sulfisoxazole

2θ	$d(\text{\AA})^*$	I/I_0^{**}	2θ	$d(\text{\AA})^*$	I/I_0^{**}
12.09	7.32	100	33.76	2.65	6
13.23	6.69	31	34.32	2.61	6
14.28	6.20	6	35.00	2.56	1
15.22	5.82	26	35.26	2.55	1
16.32	5.43	4	36.04	2.49	6
16.94	5.23	15	36.52	2.46	5
17.40	5.10	41	37.82	2.38	2
18.56	4.78	14	38.06	2.36	2
19.48	4.56	14	38.44	2.34	2
20.26	4.38	21	39.16	2.30	7
20.64	4.30	36	39.78	2.27	3
21.76	4.08	13	40.62	2.22	4
22.86	3.89	96	42.12	2.15	7
23.46	3.79	8	43.56	2.08	3
23.74	3.75	5	43.94	2.06	1
24.45	3.64	19	44.66	2.03	4
25.68	3.47	8	45.30	2.00	1
26.62	3.35	65	45.90	1.98	1
27.18	3.28	14	46.40	1.96	2
27.83	3.21	9	47.02	1.93	4
28.26	3.16	2	47.99	1.90	3
29.52	3.03	13	48.76	1.87	2
30.14	2.97	5	49.00	1.86	1
30.86	2.90	2	49.70	1.83	1
31.36	2.85	3	50.14	1.82	1
31.74	2.82	2	50.73	1.80	1
32.20	2.78	2			
33.00	2.71	6			
33.20	2.70	6			

* d - (interplanar distance) $\frac{n\lambda}{2 \sin \theta}$

** I/I_0 = relative intensity (based on highest intensity of 1.00)

2.12 Dissociation Constant

The pK_a for sulfisoxazole has been determined spectrophotometrically (11) and by the titration of

sulfisoxazole in an excess of 0.1N NaOH with 0.1N HCl to be 5.0 (12,13).

3. Synthesis

Sulfisoxazole may be prepared by the reaction scheme shown in Figure 7. 3,4-Dimethyl-5-aminoisoxazole is reacted with N-acetyl-p-aminobenzene sulfonyl chloride. The acetyl group is then cleaved to yield sulfisoxazole (10).

4. Stability Degradation

When 2% solutions of sulfisoxazole are prepared in ethanol:water (1:1) and ethanol:0.1N NaOH (1:1) and refluxed for one hour, no decomposition was observed by thin-layer chromatography (3). When a 2% solution of sulfisoxazole in ethanol:0.1N HCl (1:1) was refluxed for one hour slight decomposition to 3,4-dimethyl-5-aminoisoxazole and sulfanilamide was observed (3). Pure sulfisoxazole has been found to be stable when subjected to a temperature of 105°C for 5 days (3). In tablet form, sulfisoxazole is stable for 5 years (14).

5. Drug Metabolic Products and Pharmacokinetics

Sulfisoxazole is metabolized to its N₄-acetyl derivative which is the major metabolite found in human urine. Sulfisoxazole is ultimately eliminated from the body solely by means of urinary excretion with a mean of 54 percent of the dose excreted as the "free" drug and the remainder as the N₄-acetylated biotransformation product (15).

A pharmacokinetic profile of sulfisoxazole was determined from data obtained from I.V. administration of the drug in humans. The data suggested the use of a two compartment open system model (16). The excellent agreement between the simulated and experimental data reflects the reliability of the assumption of pseudo first order kinetics for all processes (15).

6. Methods of Analysis

6.1 Elemental Analysis

The results from the elemental analysis are listed in Table V (17).

Figure 7

Synthesis of Sulfisoxazole

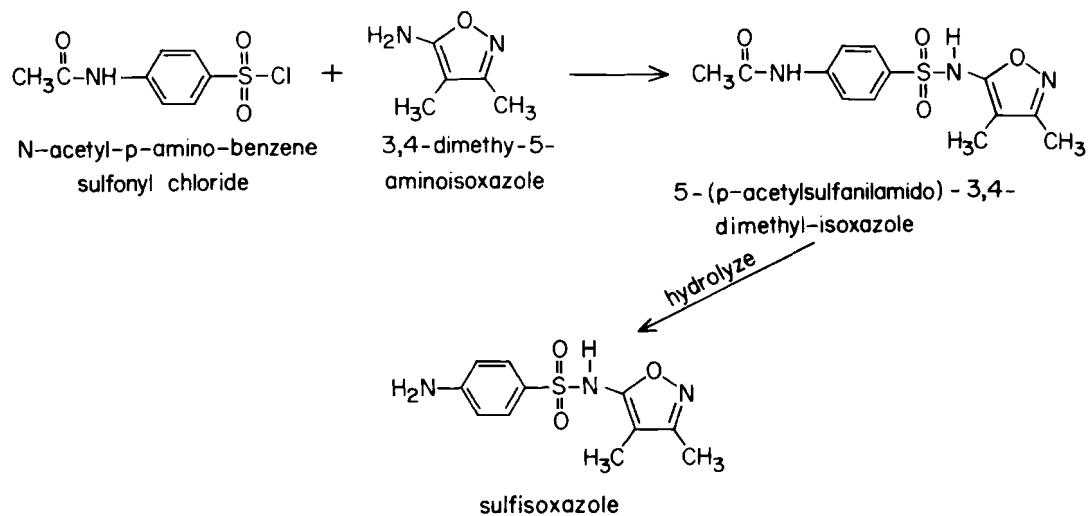


Table V

Elemental Analysis of Sulfisoxazole

<u>Element</u>	<u>% Theory</u>	<u>% Found</u>
C	49.43	49.32
H	4.90	4.81
N	15.72	15.74
S	12.00	12.07

6.2 Phase Solubility Analysis

Phase solubility analysis may be carried out using ethyl acetate as the solvent. A typical example is shown in Figure 8 which also lists the conditions under which the analysis was carried out (8).

6.3 Thin Layer Chromatographic Analysis (TLC)

TLC systems have been published which can be used to separate sulfisoxazole from its metabolites and other sulfonamides. In the system reported by Karpitschka (18) the sample is spotted on a Kieselgel G plate and developed in the solvent, chloroform:n-butanol:petroleum ether (1:1:1). Sulfisoxazole has a R_f of 0.51. The TLC system reported by Wollish et al. (19) utilizes a Silica Gel G plate and chloroform:heptane:ethanol (1:1:1) as the developing solvent. In this system sulfisoxazole has a R_f of 0.7.

6.4 Direct Spectrophotometric Analysis

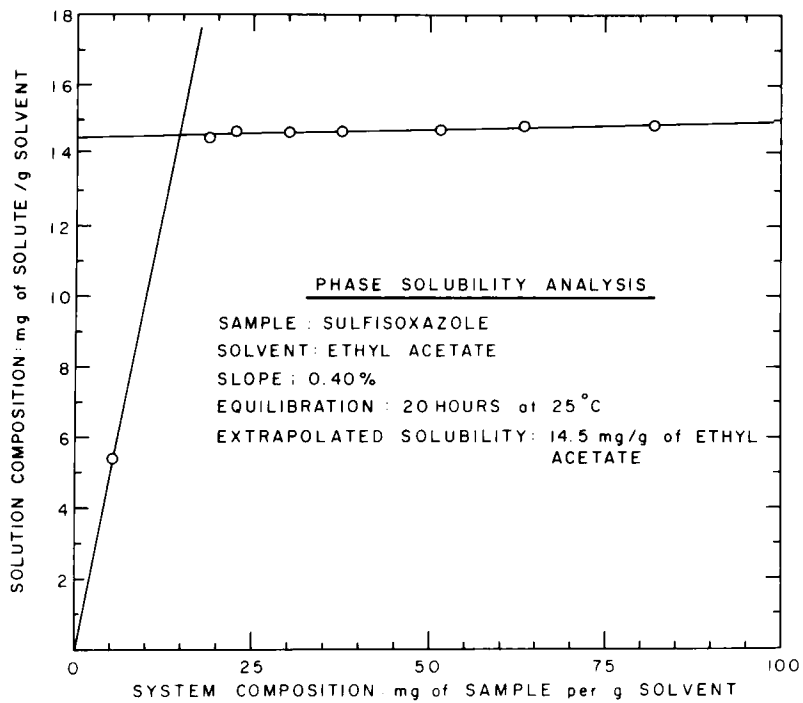
Direct spectrophotometry is not often used for the analysis of sulfisoxazole because other sulfonamides tend to interfere. However, in the absence of any interferences, the λ_{\max} at 253 nm in pH 7.5 phosphate buffer may be used for direct spectrophotometric analysis.

6.5 Colorimetric Analysis

The colorimetric analysis of sulfisoxazole may be accomplished by diazotization and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride (Bratton-Marshall Reagent). The resultant purple color is measured at about 550 nm (20).

SULFISOXAZOLE

Figure 8



6.6 Fluorimetric Analysis

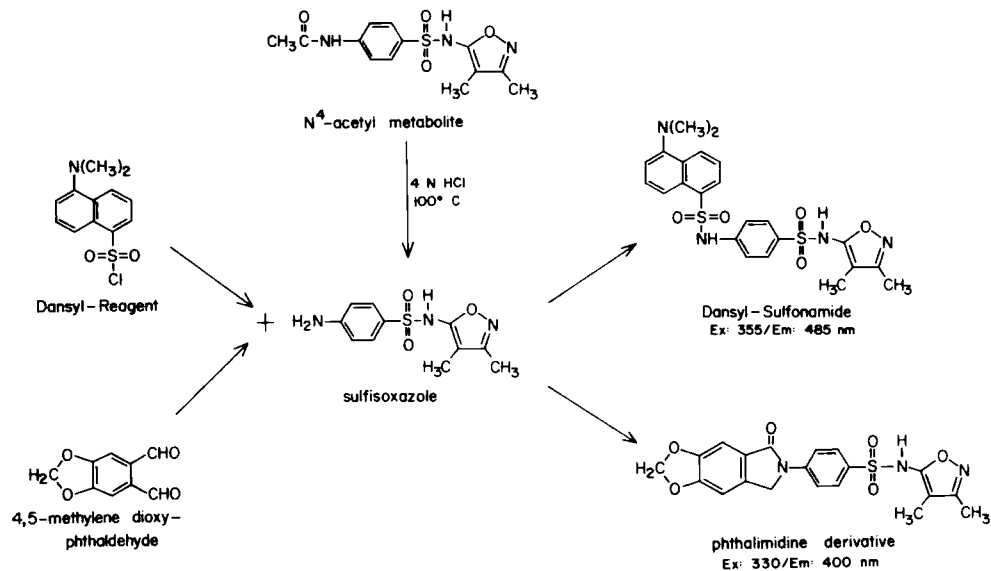
Fluorescence analysis has been used to determine the concentration of sulfisoxazole in plasma (21). For this purpose detectable levels in the range of 1 μg per ml of plasma are needed. In order to achieve this sensitivity it is necessary to make a fluorescent derivative of sulfisoxazole. One such derivative is the highly fluorescent "Dansylsulfonamide" which is formed by the reaction shown in Figure 9. The fluorescence yield of this compound is sufficient to measure about 1 μg of sulfisoxazole per ml of plasma. An even more sensitive derivative is formed when sulfisoxazole is reacted with 4,5-methylene-dioxy-phthalaldehyde to form a phthalimidine derivative (Figure 9). The sensitivity limit for this derivative is from 1×10^{-8} to 1×10^{-10} mole/ml (22).

6.7 Titrimetric Analysis

The titration with sodium methoxide is the method of choice to assay sulfisoxazole (6). The sample is dissolved in dimethylformamide, thymol blue is added, and the titration with 0.1N NaOCH_3 to a blue end-point is carried out. A blank determination is performed and any necessary correction made. Each ml of 0.1N NaOCH_3 is equivalent to 26.73 mg of sulfisoxazole.

Figure 9

Fluorescence Derivatives of Sulfisoxazole



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TRICLOBISONIUM CHLORIDE

Bruce C. Rudy and Bernard Z. Senkowski

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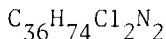
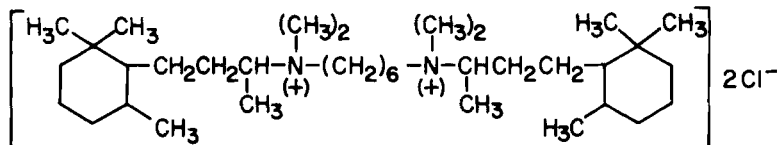
Analytical Profile - Triclobisonium Chloride

1. Description
 - 1.1 Name, Formula, Molecular Weight
 - 1.2 Appearance, Color, Odor
2. Physical Properties
 - 2.1 Infrared Spectrum
 - 2.2 Nuclear Magnetic Resonance Spectrum
 - 2.3 Ultraviolet Spectrum
 - 2.4 Fluorescence Spectrum
 - 2.5 Mass Spectrum
 - 2.6 Optical Rotation
 - 2.7 Melting Range
 - 2.8 Differential Scanning Calorimetry
 - 2.9 Solubility
 - 2.10 X-ray Crystal Properties
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4. Stability Degradation
5. Drug Metabolic Products
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 - 6.1 Elemental Analysis
 - 6.2 Thin Layer Chromatographic Analysis
 - 6.3 Colorimetric Analysis
 - 6.4 Titrimetric Analysis
7. References

1. Description

1.1 Name, Formula, Molecular Weight

Triclobisonium chloride is hexamethylenebis [dimethyl[1-methyl-3-(2,2,6-trimethylcyclohexyl)propyl] ammonium]dichloride.



Molecular Weight: 605.91

1.2 Appearance, Color, Odor

Triclobisonium chloride occurs as a white or nearly white, practically odorless, crystalline powder.

2. Physical Properties

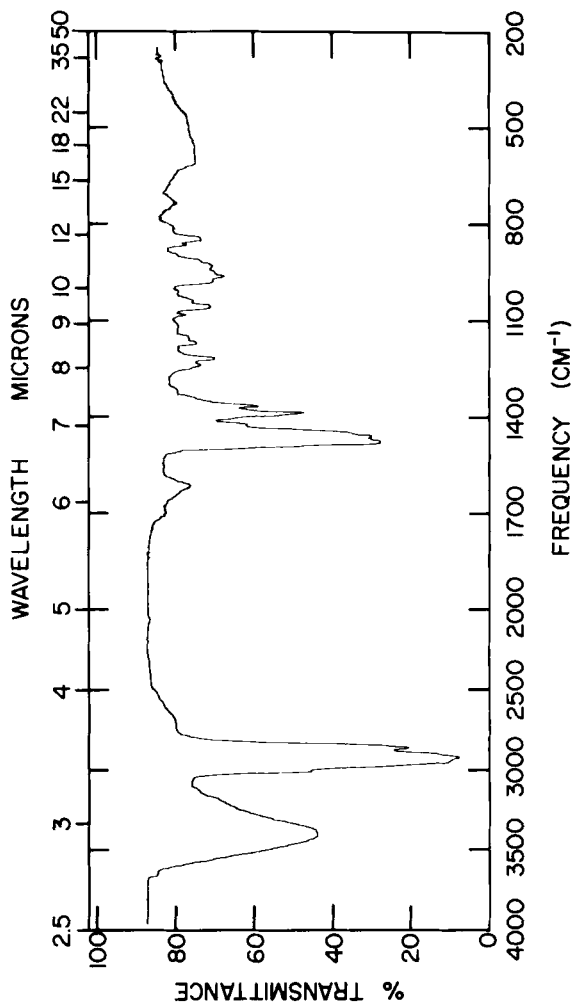
2.1 Infrared Spectrum

The infrared spectrum of triclobisonium chloride is presented in Figure 1. The spectrum was measured with a Perkin-Elmer 621 Spectrophotometer on a KBr pellet containing 1.5 mg triclobisonium chloride/300 mg of KBr. The bands at 2960 and 2860 cm^{-1} are attributed to aliphatic C-H stretching and the bands at 1474 and 1382 cm^{-1} to C-H deformation vibrations (1).

2.2 Nuclear Magnetic Resonance Spectrum (NMR)

The spectrum shown in Figure 2 was obtained on a Jeol 60 MHz NMR by dissolving 57.2 mg of triclobisonium chloride in 0.5 ml of CDCl_3 containing tetramethylsilane as an internal reference (2). Due to the many nearly equivalent protons in this molecule, the NMR spectrum consists of broad bands in the 0 to 4 ppm region. The spectral assignments are given in Table I (2).

Figure 1
Infrared Spectrum of Triclobisonium Chloride



TRICLOBISONIUM CHLORIDE

Figure 2
NMR Spectrum of Triclobisonium Chloride

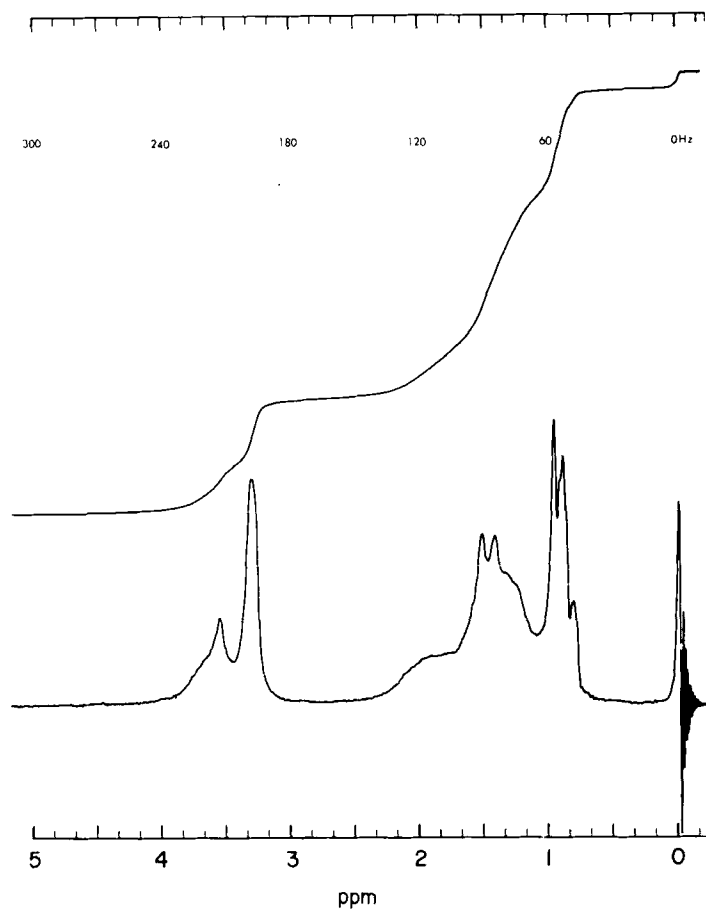


Table I

NMR Assignments for Triclobisonium Chloride

<u>Type Proton</u>	<u>Approx. Chemical Shift (ppm)</u>
CH ₃ 's on rings	0.80-0.95
CH ₃ 's on chain	1.47
CH ₂ 's & CH's in rings and chain (not adjacent to nitrogens)	1.05-2.30
CH ₃ 's on nitrogens	3.30
CH ₂ 's & CH's adjacent to nitrogens	3.45-3.90

2.3 Ultraviolet Spectrum (UV)

Triclobisonium chloride exhibits no maxima or minima in an aqueous solution (1 mg/ml) from 700 to 210 nm.

2.4 Fluorescence Spectrum

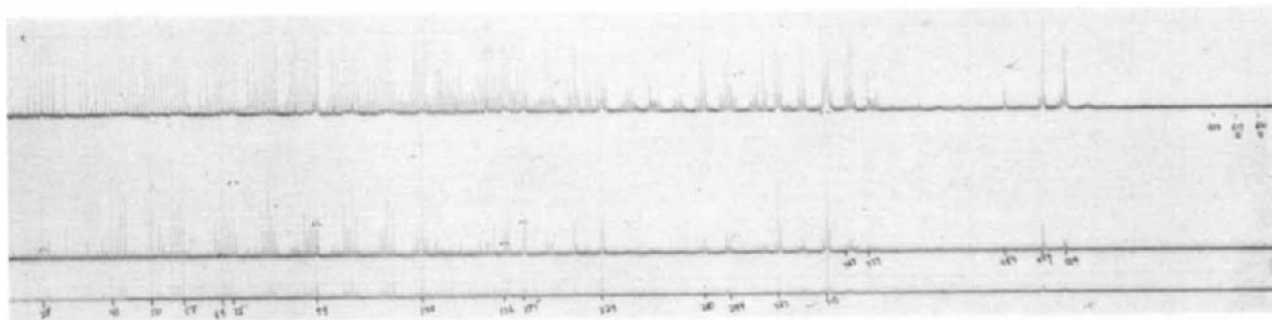
Triclobisonium chloride exhibits no fluorescence when irradiated with white light. (3)

2.5 Mass Spectrum

The mass spectrum of triclobisonium chloride shown in Figure 3 was obtained from a CEC 21-110 mass spectrometer with an ionizing energy of 70 eV (4).

Weak peaks were observed up to m/e 616 in the low resolution spectrum. Since quaternary ammonium salts are not sufficiently volatile, the mass spectrum obtained is primarily that of the thermal degradation products. In this case the main product observed had a molecular weight of 504, $C_{34}H_{68}N_2$ by high resolution analysis, which corresponds to triclobisonium chloride minus 2 CH_3Cl 's. The low resolution spectrum also contained a peak at m/e 518 probably due to the loss of HCl plus CH_3Cl . The component of molecular weight 504 gives rise to the base peak at m/e 351 and also m/e 225 via the usual cleavage β to the amino group. The peak at m/e 186, $C_{11}H_{26}N_2$, is probably a fragment from the center part of the molecule (4).

Figure 3
Mass Spectrum of Triclobisonium Chloride



TRICLOBISONIUM CHLORIDE

2.6 Optical Rotation

Triclobisonium chloride exhibits no optical activity.

2.7 Melting Range

Triclobisonium chloride decomposes on melting. The decomposition range depends on the rate of heating. When the NF Class I procedure is used (5), triclobisonium chloride melts with decomposition between 243 and 253°C.

2.8 Differential Scanning Calorimetry (DSC)

The DSC scan for triclobisonium chloride, shown in Figure 4, was obtained using a Perkin-Elmer DSC-1B Calorimeter. The sample pan contained a small hole in the top to allow the decomposition products formed to escape. With a temperature program of 10°C/min., an endotherm was observed starting at 253.4°C which is due to the melt and/or decomposition of the molecule (6). Due to the nature of this peak no ΔH_f was calculated.

2.9 Solubility

The solubility data for triclobisonium chloride obtained at 25°C are given in Table II (7).

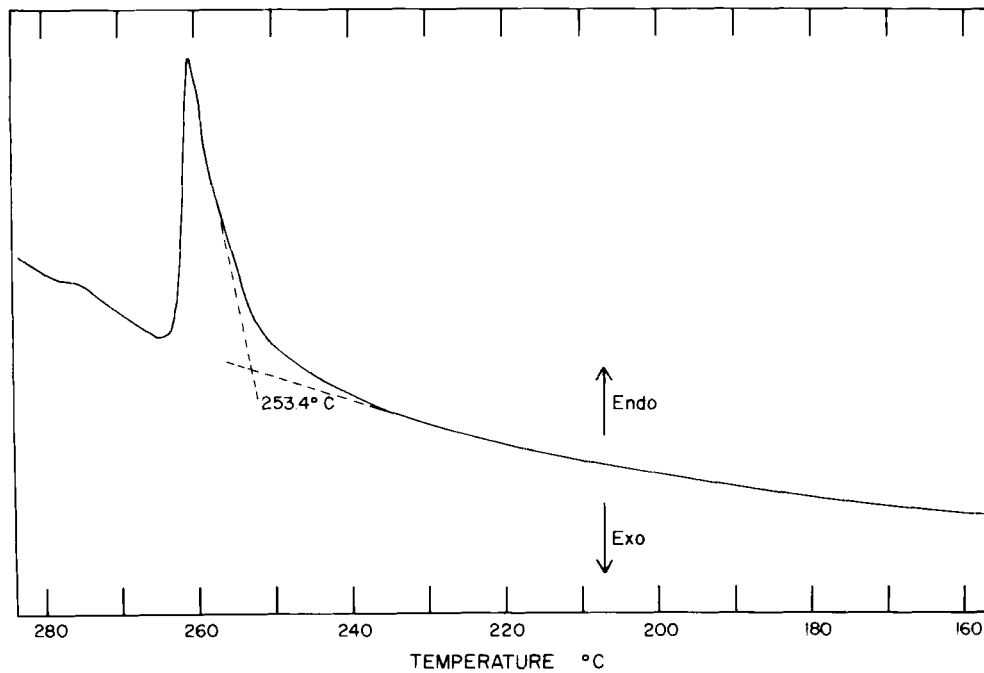
Table II

Solubility of Triclobisonium Chloride
in Different Solvents

<u>Solvent</u>	<u>Solubility (mg/ml)</u>
3A alcohol	$> 5.0 \times 10^2$
benzene	1.3×10
chloroform	4.2×10^2
95% ethanol	$> 5.0 \times 10^2$
ethyl ether	1.6×10^{-1}
methanol	$> 5.0 \times 10^2$
petroleum ether	$< 5.0 \times 10^{-2}$
(30°-60°)	
2-propanol	4.1×10^2
water	$> 5.0 \times 10^2$

Figure 4

D.S.C. Curve for Triclobisonium Chloride



2.10 X-ray Crystal Properties

The x-ray powder diffraction pattern of triclobisonium chloride is presented in Table III (8). The instrumental conditions are given below.

Instrumental Conditions:

General Electric Model XRD-6 Spectrogoniometer

Generator:	50 KV-12- $\frac{1}{2}$ MA
Tube target:	Copper \circ
Radiation:	Cu K = 1.542 Å
Optics:	0.1° Detector slit
	M.R. Soller slit
	3° Beam slit
	0.0007 inch Ni filter
	4° take off angle
Goniometer:	Scan at 0.2° 2 θ per minute
Detector:	Amplifier gain - 16 coarse,
	8.7 fine
	Sealed proportional counter
	tube and DC voltage at
	plateau
	Pulse height selection E _L -5
	volts; Eu - out
	Rate meter T.C. 4
	2000 C/S full scale
Recorder:	Chart speed - 1 inch/5 min.

Samples prepared by grinding at room temperature.

TABLE III

X-ray Powder Diffraction Pattern
Triclobisonium Chloride

2 θ	$d(\text{Å})^*$	I/I_o^{**}	2 θ	$d(\text{Å})^*$	I/I_o^{**}
3.98	22.2	39	23.78	3.74	19
4.44	19.9	75	24.46	3.64	17
12.44	7.12	19	26.18	3.40	6
12.92	6.85	10	26.38	3.38	22
13.52	6.55	25	27.14	3.29	22
14.74	6.01	100	28.00	3.19	15

TRICLOBISONIUM CHLORIDE

TABLE III (cont.)

X-ray Powder Diffraction Pattern
Triclobisonium Chloride

2θ	$d(\text{\AA})^*$	I/I_0^{**}	2θ	$d(\text{\AA})^*$	I/I_0^{**}
15.40	5.75	6	28.62	3.12	19
15.94	5.56	28	29.42	3.04	28
16.56	5.35	6	30.56	2.93	11
17.16	5.17	14	31.04	2.88	15
17.78	4.99	67	32.10	2.79	11
19.92	4.46	18	32.76	2.73	13
20.60	4.31	4	34.96	2.57	8
21.60	4.11	13	35.74	2.51	8
22.00	4.04	19	41.16	2.19	8
23.36	3.81	25	45.12	2.01	8

* $d = (\text{interplanar distance}) = \frac{n\lambda}{2 \sin \theta}$

** $I/I_0 = \text{relative intensity (based on highest intensity of 1.00)}$

3. Synthesis

Triclobisonium chloride may be prepared by the reaction scheme shown in Figure 5. A comprehensive review of the synthesis of several symmetrical bis-quaternary amines derived from β -ionone has been published by Teitel (9).

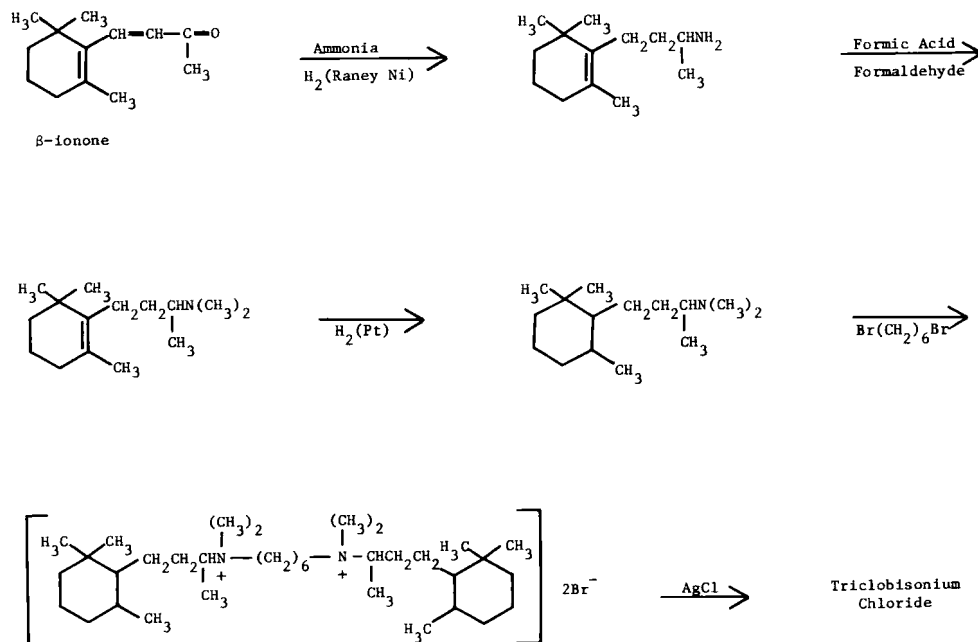
4. Stability Degradation

Triclobisonium chloride was found to be stable to light and oxygen in both slightly acidic (pH 3-7) and slightly basic (pH 7-9) solutions. In strongly alkaline solutions the compound decomposes to yield tertiary amines (10). In the cream or ointment, the triclobisonium chloride is stable for a minimum of 4 years. The bulk substance is stable when stored at room temperature under anhydrous conditions. Decomposition occurs when it is subjected to elevated temperatures as can be detected by discoloration and odor of the powder.

5. Drug Metabolic Products

Triclobisonium chloride is an antibacterial agent used for the treatment and/or prevention of local infections. Since it is not administered internally, triclobisonium

Figure 5
Synthesis of Triclobisonium Chloride(9)



chloride is not metabolized by the body.

6. Methods of Analysis

6.1 Elemental Analysis

The results from the elemental analysis are listed in Table IV (11).

TABLE IV

Elemental Analysis of Triclobisonium Chloride

<u>Element</u>	<u>% Theory</u>	<u>% Found</u>
C	71.36	69.29
H	12.31	12.30
N	4.62	4.57
Cl	11.70	11.46

6.2 Thin Layer Chromatographic Analysis (TLC)

The following TLC procedure may be used to identify triclobisonium chloride. Spot 500 mcg of triclobisonium chloride which was dissolved in methanol on a silica gel G plate and subject it to ascending chromatography in a paper lined, saturated tank using a mixture of acetone:5% KCl: benzene (80:40:1) as the solvent. After developing the plate at least 15 cm, it is air dried and sprayed with iodine-modified Dragendorff reagent to visualize the spot. The triclobisonium chloride will appear as a brown spot about R_f 0.5 (12).

6.3 Colorimetric Analysis

6.31 Ion Pair Extraction of Bromocresol Green Complex

After dissolution of the triclobisonium chloride cream, the triclobisonium chloride is complexed with bromocresol green and this complex is extracted into a chloroform solution. The absorbance of this solution, as well as the absorbance of the complex similarly prepared from reference standard triclobisonium, is read at 630 nm and the amount of triclobisonium chloride present in the cream is calculated (13).

6.32 Ion-Pair Extraction of Bromothymol Blue Complex

For the triclobisonium chloride ointment bromothymol blue is used to form the ion-pair complex. The procedure is similar to that for the cream except polyethylene glycol 4000 and polyethylene glycol 400 are added to the reference standard triclobisonium chloride solution and also to a blank. After extraction of the complex from a basic aqueous solution, the absorbance is read at the 384 nm maximum for the sample, standard, and the blank. These values are used to calculate the amount of triclobisonium in the ointment (13).

6.4 Titrimetric Analysis

The non-aqueous titration as described in the NF XIII is the method of choice for the analysis of bulk triclobisonium chloride (13). An accurately weighed 1 gm sample is dissolved in 80 ml of glacial acetic acid. Fifteen milliliters of mercuric acetate and 4 drops of crystal violet are added and the triclobisonium chloride is titrated to a blue-green end-point with 0.1N HClO_4 in glacial acetic acid. A blank titration is performed and any necessary correction made. Each ml of 0.1N HClO_4 is equivalent to 30.30 mg of $\text{C}_{36}\text{H}_{74}\text{Cl}_2\text{N}_2$ (13).

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TRIFLUPROMAZINE HYDROCHLORIDE

Klaus Florey

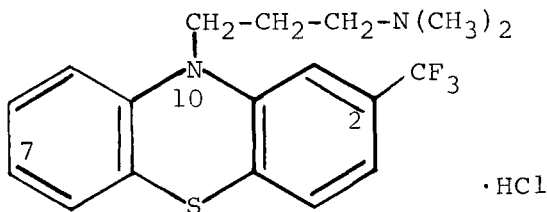
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1. Description

1.1 Name, Formula, Molecular Weight

Triflupromazine Hydrochloride is 10- γ -(Dimethylamino)-propyl-2-trifluoromethylphenothiazine hydrochloride; also 2-(Trifluoromethyl)-10-(γ -dimethylaminopropyl)phenothiazine; SQ 4703.


 $C_{18}H_{19}F_3N_2S \cdot HCl$

Mol. wt. 388.89

1.2 Appearance, Color, Odor.

Triflupromazine hydrochloride occurs as a white to pale tan, crystalline powder, with a slight characteristic odor.

2. Physical Properties

2.1 Infrared Spectra

The infrared spectra of Squibb Standard # 48244-003 are presented in figures 1 and 2.¹ The infrared spectrum of the free base and a discussion of spectra-structure

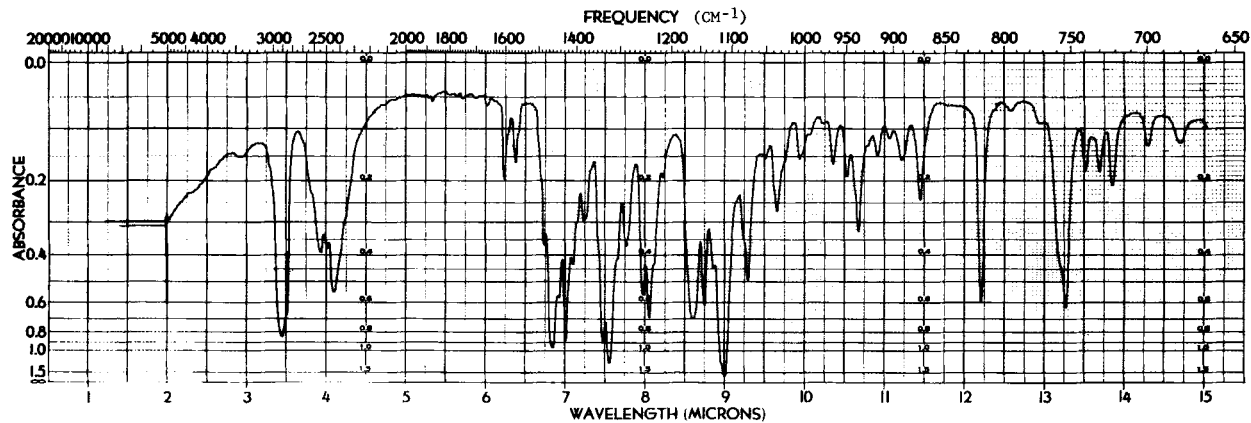


Figure 1. I.R. Spectrum of Triflupromazine Hydrochloride. Squibb Standard #48241-003. Mineral oil mull. Instrument: Perkin-Elmer 21.

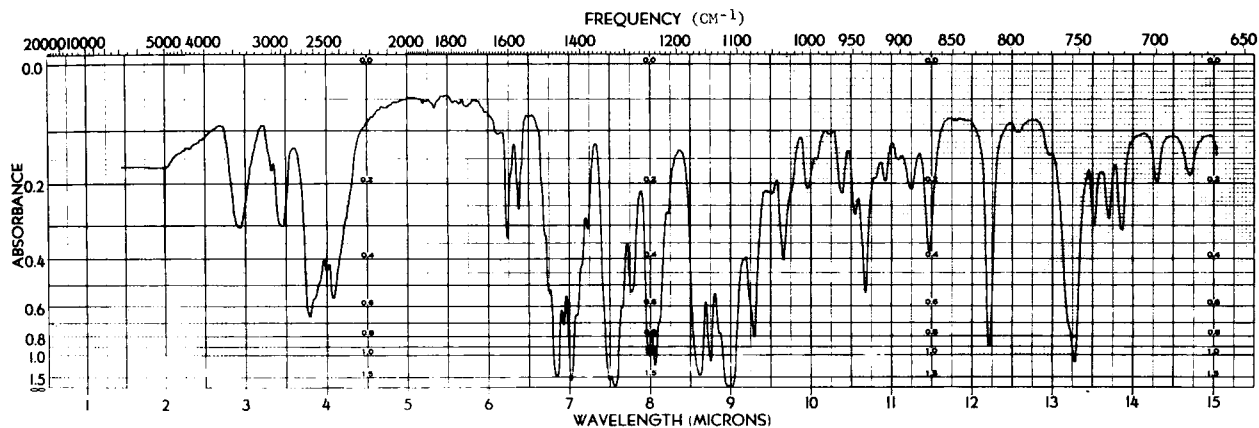
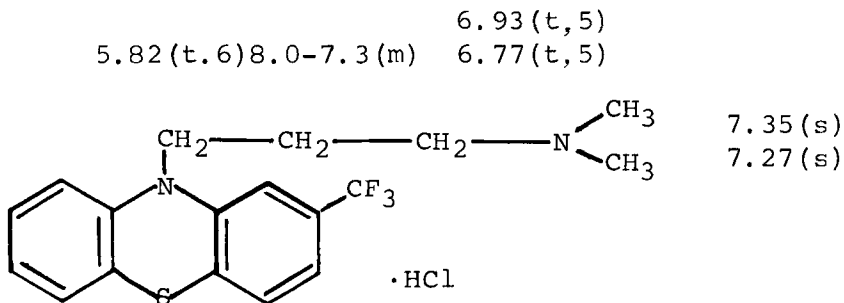


Figure 2. I.R. Spectrum of Triflupromazine Hydrochloride. Squibb Standard #48241-003 in KBr pellet from methanol. Instrument: Perkin-Elmer 21.

correlation of phenothiazines have been presented.^{2,3} The spectra published by Sammul, et al⁴ are in essential agreement with those presented here.

2.2 Nuclear Magnetic Resonance Spectrum

The NMR spectrum of triflupromazine hydrochloride is presented in Figure 3.⁶⁷ The N-methyl proton resonances are seen as two singlets. The methylene proton resonances of the two methyl groups on the protonated nitrogen appear as two sets of triplets because of rotational non-equivalence. The following assignments in tau values are consistent with the structure:



Aromatic protons: 3.2- 2.5 τ

The NMR spectrum of the free base has been presented and discussed.³

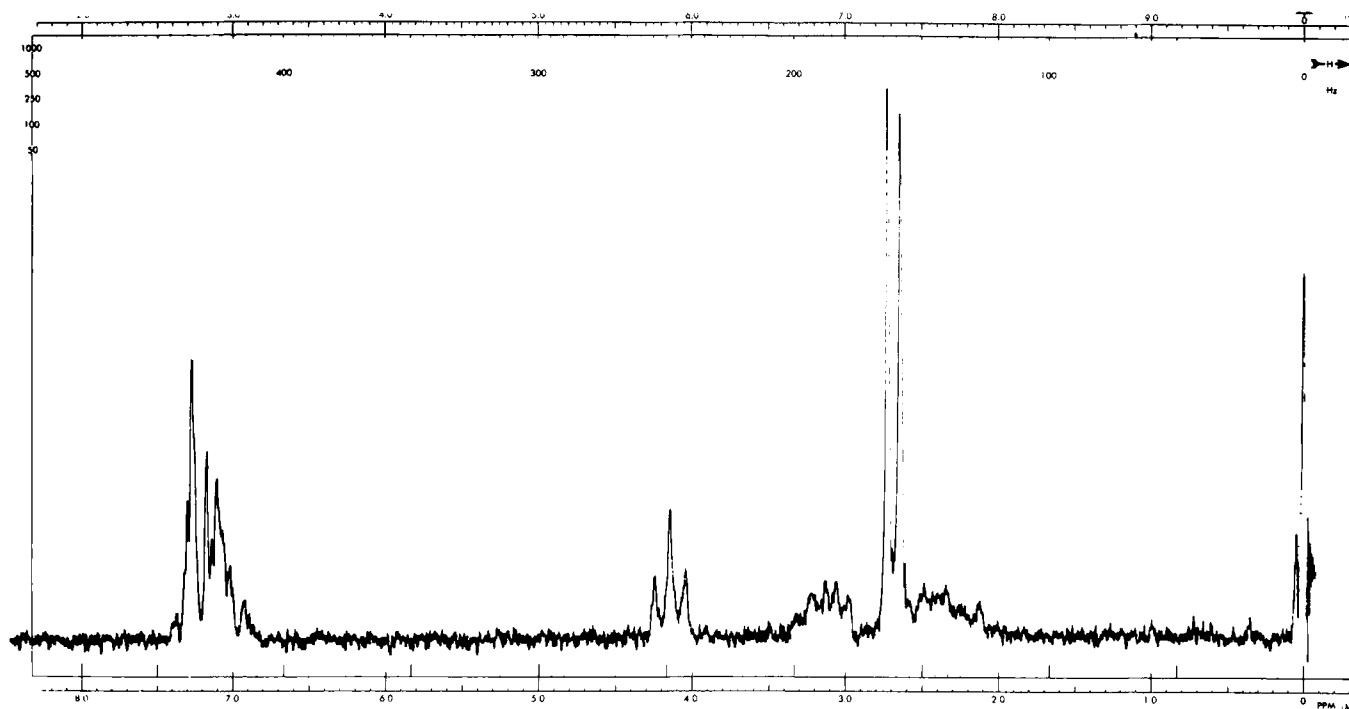


Fig. 3 NMR Spectrum of Triflupromazine Hydrochloride (Squibb Batch # 48244-003 in CDCl_3 , containing tetramethylsilane as an internal reference. Instrument: Varian A-60.

2.3 Ultraviolet Spectrum

The following U.V. data have been recorded:

1. in methanol¹ (Instrument Cary 15)

max 260 nm $E_{1\text{cm}}^{1\%}$ 869

max 308 nm $E_{1\text{cm}}^{1\%}$ 92.8

2. in 95% ethanol³ (Instrument Cary 14)

max 258 nm ϵ 34,000

max 308 nm ϵ 3,700

min 224 nm ϵ 10,000

min 280 nm ϵ 1,600

2.4 Mass Spectrum

The low resolution mass spectrum of triflupromazine is presented in figure 4.

The following fragmentation pattern⁵ presented in figure 5 is consistent with the mass spectrum and in agreement with the results of an independent investigation.⁶

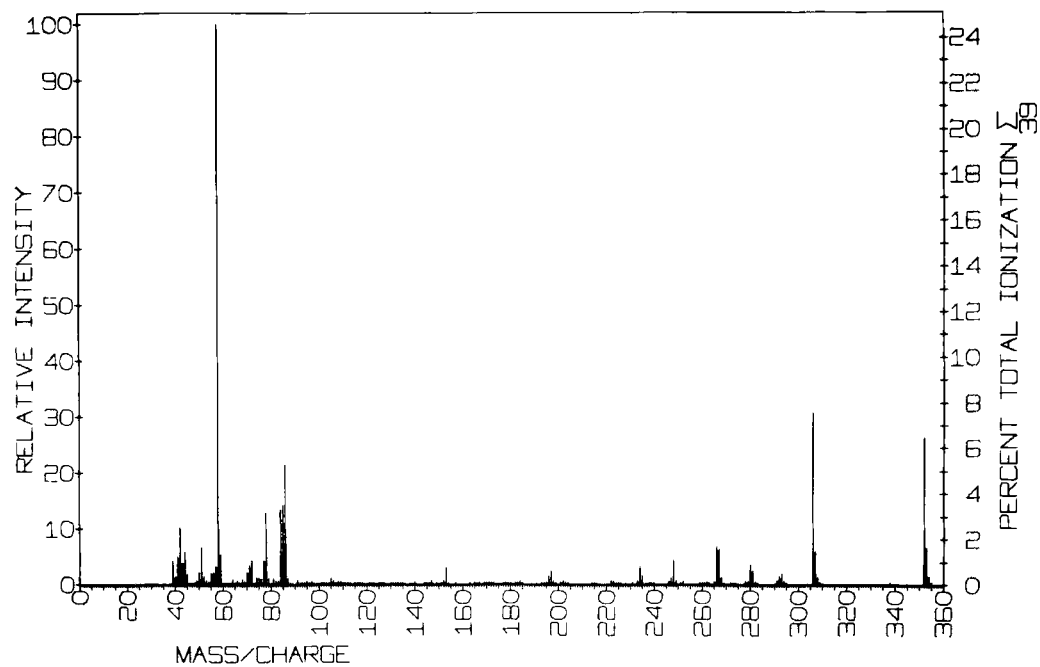


Fig. 4 Low Resolution Mass Spectrum of Triflupromazine.
Instrument: MS 902.

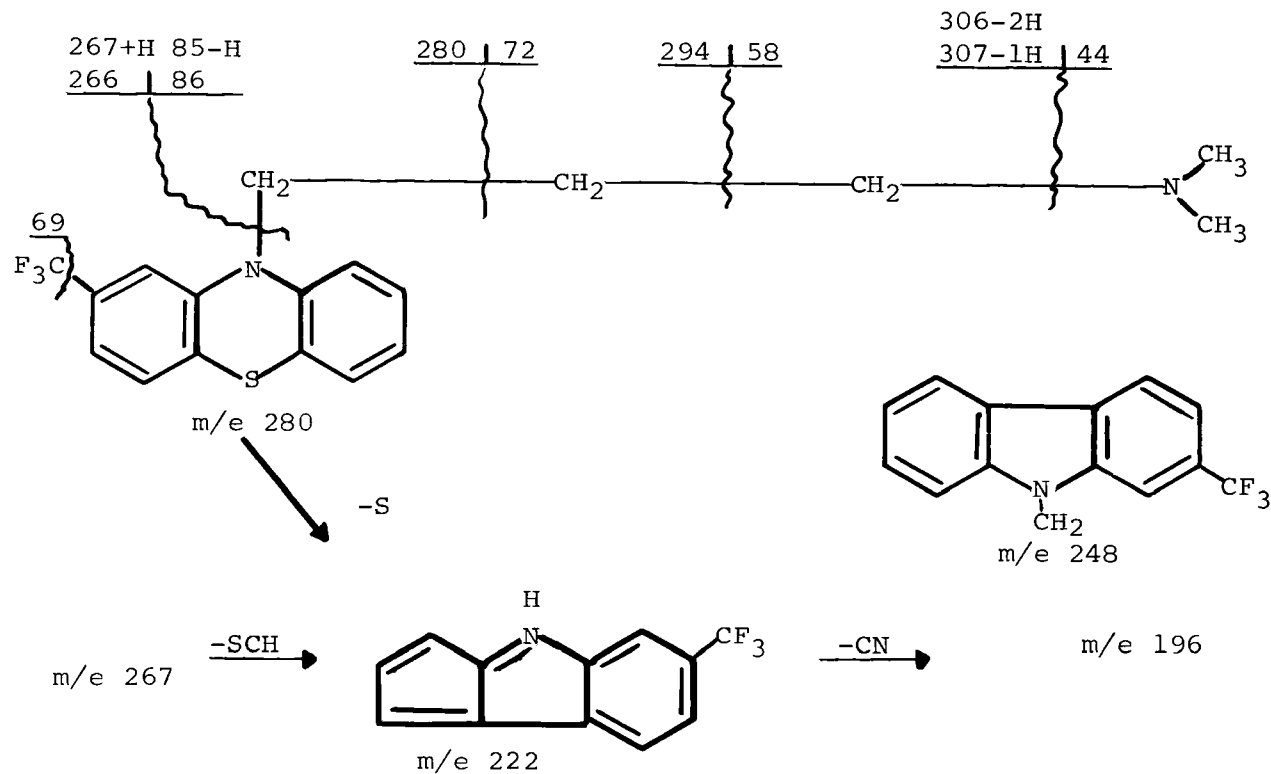


Figure 5. Fragmentation Pattern of Triflupromazine.

2.5 pK, pH

The pK_a of the hydrochloride was determined to be approximately 6.5⁷, the pK_b of the free base to be 4.59.⁸ The pH of a 2% solution of a recent standard was found to be 4.7.¹ The pK_a of the free base was determined to be 9.2.⁹

2.6 Melting Range

The following melting (decomposition) ranges have been reported ($^{\circ}C$).

173-174 (Recrystallized from dry chlorobenzene and acetonitrile)¹⁰

176-177¹¹

2.7 Differential Thermal Analysis

Differential Thermal Analysis of Squibb Batch AR-IX-113E showed an endotherm at 174 $^{\circ}C$.¹⁶

2.8 Thermogravimetric Analysis

Thermogravimetric analysis of Squibb Batch AR-IX-113E gave a weight loss of 0.1%.¹⁰

2.9 Solubility

Triflupromazine hydrochloride is very water soluble and hygroscopic.¹²

solubility in

water: >1g/1 ml
ethanol: >1g/1 ml
chloroform: 0.6g/1 ml
ether: insoluble

The solubility of the free base in water was determined to be 5 μ M.⁹

2.10 Crystal Properties

For forensic identification a picture of triflupromazine crystals from 95% ethanol has been published with those of other phenothiazines¹³.

An X-ray powder diffraction pattern of triflupromazine hydrochloride is presented in table I.¹⁴

Table I

$d(\text{\AA})^{\circ}$ *	$1/100^{\circ}$ **
14.75	50
8.05	50
7.05	35
5.05	60
4.75	100
4.50	25
4.25	15
4.10	80
3.85	40
3.75	40
3.55	40
3.45	10
3.30	10
3.20	15

* d = interplanar distance (\AA)

** Based on highest intensity of
100 Cu $k\alpha$ radiation;
= 1.5418 \AA

The X-ray powder diffraction pattern of the picrate has been used for identification in formulations.¹⁴

3. Synthesis

Triflupromazine is prepared by addition of the dimethylaminopropyl sidechain to the phenothiazine nucleus (II)^{10,11} or its N-acetyl derivative⁶⁵ (III) and conversion of the base to the hydrochloride (I). Diazotization of the 7-aminoanalogue (IV) also yields I.¹⁵

4. Stability - Degradation

Triflupromazine hydrochloride is hygroscopic. When moisture is excluded it is stable in the

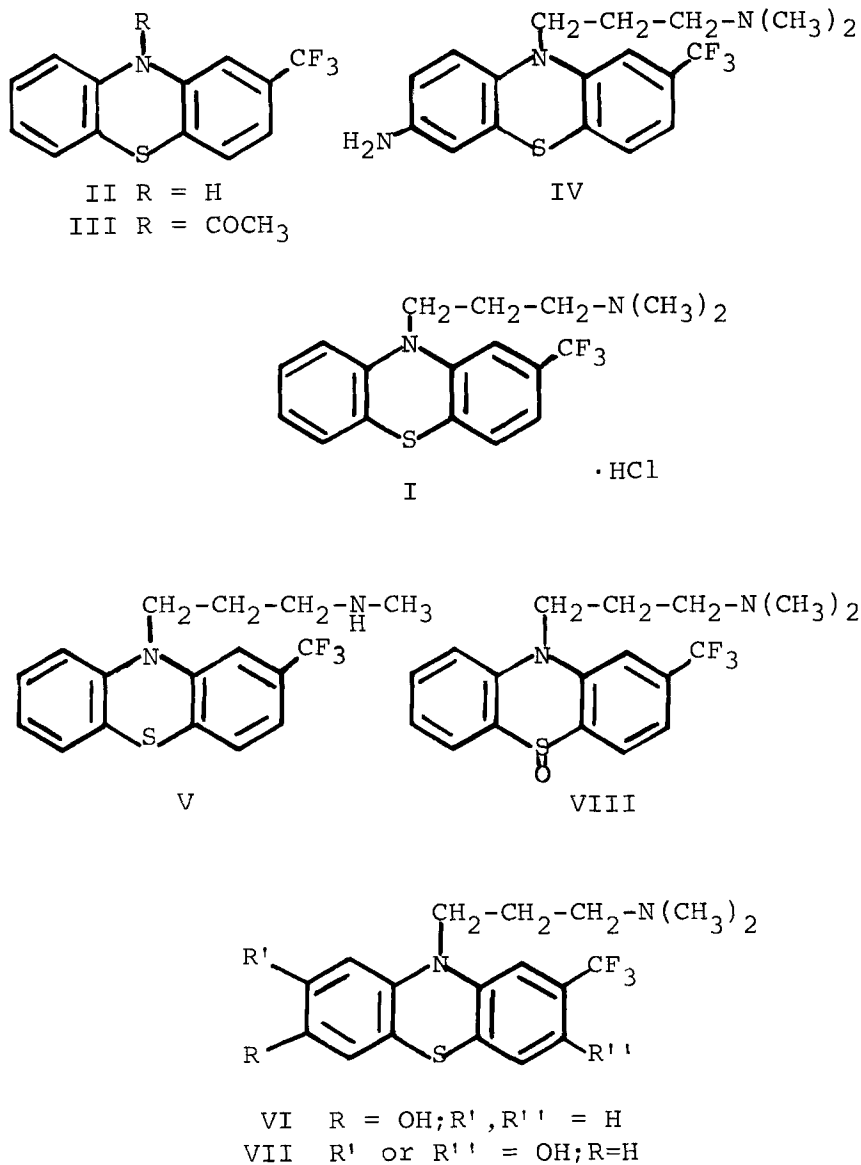


Fig. 6 Synthetic and Degradative Pathways for Triflupromazine Hydrochloride.

crystalline state, but discolors rapidly when exposed to ultraviolet radiation.⁶⁶ This discoloration also occurs on U.V. radiation of aqueous solutions with spectral changes.⁶⁶ It is undoubtedly due to radical formation and subsequent disproportionation (see below). Thin-layer chromatographic spots on silica gel are oxidized to the sulphoxide (VIII fig. 6) when exposed to air for 48-72 hours.¹⁷ Hydrogen peroxide hastened this process.¹⁷ Oxidation also proceeds through the colored semiquinone free radical (cf. 18). In phenothiazines generally these radicals undergo disproportionation. The products of oxidation are the sulfoxide or the 3-(or 7-) hydroxy derivatives. However, formation of the latter is severely inhibited in 10-substituted phenothiazines.¹⁸ So far there is no evidence for the degradative formation of 7-hydroxy-triflupromazine (VI fig. 6) which has been identified as a metabolic product¹⁹ (see Section 5). The Electron Spin Resonance spectrum of the radical has been measured and presented^{18,20,21} and was found to differ from the spectrum of the parent compound in the lack of hyperfine structure. The second order decay rate constant of the semiquinone radical of triflupromazine was found to be 870 (1/mol/min) as measured by ESR.¹⁸

5. Drug Metabolic Products

When triflupromazine was incubated with certain liver preparation in vitro, evidence was obtained for conversion to N-demethyl-triflupromazine (V fig. 6), 7-hydroxytriflupromazine (VI), traces of triflupromazine

sulphoxide (VIII) and possibly 3- or 8-hydroxytriflupromazine (VII).¹⁹

6. Methods of Analysis

6.1 Elemental Analysis:

Element	% Calc.	% Found	
		Ref. 10	Ref. 11
C ₁₈	55.59	55.70	55.33
H ₂₀	5.18	5.22	5.33
Cl	9.12	--	--
F ₃	14.66	--	--
N ₂	7.21	--	7.14
S	8.24	--	--

6.2 Gravimetric Analysis

The precipitate with silicotungstic acid has been used for the quantitative determination of triflupromazine hydrochloride in tablets.²²

6.3 Nonaqueous Titration

The nonaqueous titration of triflupromazine hydrochloride in acetone with perchloric acid in dioxane as titrant and dimethyl yellow as indicator has been described.²² It is suitable for formulation content assays. Titration with perchloric acid was employed to determine the half neutralization potentials (E-1/2) with a Fisher titrimeter in several organic solvents.⁸ The following E-1/2 values (in mv) were found: acetic acid 234, acetone 455, acetonitrile 424, isopropanol 445, nitromethane 314.

6.4 Electroanalysis

Controlled potential electrolysis has been applied for the coulometric quantitative determination of triflupromazine and other phenothiazines.²³ Depending on the oxidation potential (anode potential with respect to S.E.C. (standard calomel electrode)) and acid strength triflupromazine was oxidized to the free radical (Ox. Pot. + 0.65; 12N H₂SO₄) or the sulfoxide (Ox. Pot. + 1.05; 12N H₂SO₄). The electrochemistry of triflupromazine in acetonitrile has also been studied.²⁴ Polarographic evidence has been presented to prove the complexation of triflupromazine hydrochloride with oxygen.²⁵

6.5 Titrimetric Analysis

A titrimetric method has been reported in which triflupromazine hydrochloride was titrated visually to a colorless endpoint with ceric sulfate.²⁶ The reaction proceeds through forming a red-colored semiquinone free radical (420 nm) followed by formation of the colorless sulfoxide derivative. The method can be applied to pharmaceutical dosage forms.

6.6 Spectrophotometric Analysis

The ultraviolet absorption maximum at 255 nm has been used for a quantitative assay of triflupromazine hydrochloride in formulations.

6.7 Spectrofluorometric Analysis

Triflupromazine hydrochloride, like other phenothiazine drugs exhibits fluorescence. This phenomenon was first explored in 0.2N sulfuric acid.²⁷ The fluorescence peak was found in the range of 450 to 475 nm, shifting to slightly lower wavelength upon oxidation with potassium permanganate. As little as 0.01 to 0.05 μg of the pure substance per ml. of 0.2N sulfuric acid could be detected. Due to interference by other substances, the sensitivity in biological fluids was lower (about 0.8 $\mu\text{g}/\text{ml}$ of fluid).

In concentrated sulfuric acid, the activation maximum was found at 325 nm and the fluorescence maximum at 500 nm.²⁸ The limit of sensitivity was 0.05 $\mu\text{g}/\text{ml}$. In 50% acetic acid oxidation to the sulfoxide with 30% hydrogen peroxide gave an activation maximum at 350 m μ and a fluorescence maximum of 410 m μ .²⁹

6.8 Electrophoresis

Electrophoresis in various buffers, proposed by Wernum³⁰ was carried out on triflupromazine hydrochloride and several other phenothiazine tranquillizers.³¹ A Gordon-Misco apparatus, Whatman 3MM paper 30 cm in width, and a potential difference of 500 to 800 volt was used. Detection of spots was carried out with a 40% sulfuric acid spray (orange color) or

TRIFLUPROMAZINE HYDROCHLORIDE

fluorescence under U.V. light.
For triflupromazine hydrochloride,
anodic migration (cm) after 40 to 60
minutes was:

pH	3.3	4.7	6.0	7.2	8.0	9.3
Migration	5.9	4.8	5.3	5.5	4.6	1.8

6.9 Chromatographic Analysis

6.91 Paper

The following paper chromatographic system have been reported.

Solvent systems:	<u>Rf</u>	<u>Ref.</u>
1N sodium formate	0.41	31
1N sodium formate 90/n-propanol 10	0.50	31
1N sodium formate 90/1N ammonia 10	0.12	31
1N sodium formate 97/90% formic acid 3	0.59	31
1N sodium acetate	0.35	31
1N sodium acetate 90/n-propanol 10	0.59	31
10% sodium chloride 92/n-propanol 8	0.76	31
"Reverse Phase": Paper impregnated with glycerol tributyrate, 0.2M sodium acetate/HCl pH 4.58	----	32

Detection systems:

	<u>Color</u>	<u>Ref.</u>
40% H ₂ SO ₄ spray		
Fluorescence under U.V. light	Orange	31,32
Iodoplatinate	Bluish Yellow	31
	Dark	32

6.92 Thin-Layer

The following thin-layer chromatographic systems have been reported:

<u>Absorbent</u>	<u>Solvent System</u>	<u>R_f</u>	<u>Ref.</u>
Silica Gel	tert. butyl alcohol	0.36	31
"	90%/1N ammonia 10%		
"	n-propanol 88%/1N ammonia 12%	0.50	31
"	ether, sat. with water	0.48	31
"	70% methanol/water 30%	0.22	31
"	85% n-propanol/water 15%	0.22	31
"	n-butanol sat. with 1N ammonia	0.72	31
"	benzene-dioxane-aq. ammonia (60:35:5)	0.95	33
"	ethanol-acetic acid-water (50:30:20)	0.79	33
"	methanol-butanol (60:40)	0.40	33
"	cyclohexane-diethylamine (9:1)	0.76	34
"	chloroform-hexane (50:50)	0.48	35
"	chloroform-methanol (50:50)	0.86	35
"	chloroform-methanol (90:10)	0.54	36
"	acetone	0.38	36
"	methanol-ammonia (98:2)	0.60	36
"	chloroform-methanol-ammonia (80:20:1)	0.84	19+
"	cyclohexane-benzene-diethylamine (75:15:10)	0.57	37

TRIFLUPROMAZINE HYDROCHLORIDE

<u>Absorbent</u>	<u>Solvent System</u>	<u>Rf</u>	<u>Ref.</u>
Silica Gel [*]	cyclohexane-benzene- diethylamine(75:15:20)	0.60	36
" [*]	methanol	0.52	37
" [*]	acetone	0.48	37
" [*]	benzene-ethanol- ammonia (95:15:5)	0.74	36
" ^{**}	methanol	0.48	37
" ^{**}	95% ethanol	0.31	37
"	15% aq. ammonium acetate-methanol (20:80)	0.69	17 ^{***}
"	n-propanol-water- acetic acid	0.28	19 ⁺⁺
"	pyridine-pet.ether- methanol(1:4.5:0.1)	0.54	38
"	acetone-ethylacetate- ethanol 5:4:1 sat. with ammonium lactate buffer pH 7.	0.30	39

* prepared with 0.1M KOH

** prepared with 0.1M NaHSO₃

*** In this system, triflupromazine sulfoxide had an Rf of 0.50.

+ In this system, triflupromazine sulfoxide had an Rf of 0.70.

++ Triflupromazine sulfoxide had an Rf of 0.10.

Generally, it is important to protect plates from light during spotting and development.

The following detection systems have been used:

<u>System</u>	<u>Color Reaction</u>	<u>Ref</u>
Fluorescence under U.V. lamp	violet	31,36,19,17,35
40% sulfuric acid spray	orange	42,31
Potassium iodoplatinate		52,17
5% ferric chloride, 20% perchloric acid	{ flesh or pink	
50% nitric acid (5:45:50). (FPN; (Forrest reagent)		
Iodine		36,34,37 37
1% potassium permanganate		
Dragendorff reagent		37
5% p-dimethylaminobenzaldehyde in 18N sulfuric acid	cameo	36
Furfural reagent	pink	36
Folin-Ciocalteu reagent	cameo	36
1% ammonium vanadate in 10 ml.conc. sulfuric acid	flesh	36,37
5% cinnamic aldehyde and 5% HCl in ethanol	cameo	37
Sulfuric acid-formaldehyde		39
50% aq.sulfuric acid in ethanol(4:1)	orange	42,31

6.93 Gas-liquid

Triflupromazine was well separated from other phenothiazine tranquilizers on a 80-100 mesh Gas-Chrom S glass column coated with 2% SE-30. Retention time relative to chlorpromazine at a column temp. of 205° was 0.46.⁴⁰ Similar results were obtained on a 60-80 mesh Diatoport copper column coated with 5% SE-30.^{41,42} In the same system triflupromazine was separated from its sulphoxide.¹⁷ Another system used was a 100-120 mesh silanized Gas-Chrom P glass column coated with 1% Hi-Eff-8B (cyclohexanedimethanol succinate).⁴³ The identification of triflupromazine and other phenothiazine tranquilizers by gas chromatography of their pyrolysis products has also been reported.⁴⁴

7. Identification and Determination in Body Fluids and Tissues

Many of the references cited in previous sections concern methods devised to identify and determine triflupromazine hydrochloride and other phenothiazines for pharmacological, toxicological and forensic purposes (cf. 45). These methods can be classified as follows:

References

Color reaction:	46,47,48,49,50,51,52,53,54
Separation schemes:	55,50,51,52
Spectrofluorometry:	27,29,28,56
Electrophoresis:	31
Paper chromatography:	31,32
Thin-layer chromatography:	50,33,31,13,36,19,37,35, 39,68
Gas-liquid chromato- graphy:	17,43,69
X-ray diffraction bands:	61,15
Micro crystalline identification:	57,58
Photomicrography:	13

8. Miscellaneous

The following parameters of triflu-
promazine hydrochloride and other
phenathiazine have been studied:
Adsorption by solids such as talc,
kaolin and activated charcoal⁵⁹,
surface activity at the air-solution
interface^{60,61}, oil-water
partitioning⁶², photo induced inter-
action with a lecithin monomolecular
film⁶³, and interaction with bovine
serum albumin⁶⁴.

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TRIMETHOBENZAMIDE HYDROCHLORIDE

Kenneth W. Blessel, Bruce C. Rudy, and Bernard Z. Senkowski

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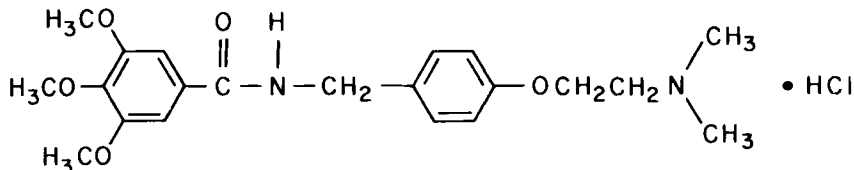
Analytical Profile - Trimethobenzamide Hydrochloride

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1. Description

1.1 Name, Formula, Molecular Weight

Trimethobenzamide hydrochloride is N-[p-[2-(dimethyl amino)ethoxy]benzyl]-3,4,5-trimethoxybenzamide monohydrochloride.



$C_{21}H_{28}N_2O_5 \cdot HCl$

Molecular Weight: 424.93

1.2 Appearance, Color, Odor

Trimethobenzamide hydrochloride is a white crystalline powder with a slight phenolic odor.

2. Physical Properties

2.1 Infrared Spectrum

The infrared spectrum of a sample of reference standard trimethobenzamide hydrochloride is shown in Figure 1 (1). The spectrum was recorded on a sample of 1.0 mg of trimethobenzamide hydrochloride dispersed in 300 mg of KBr, using a Perkin Elmer 621 Spectrophotometer. The following assignments have been made (1):

Band	Assignment
3308 cm^{-1}	N-H stretch
2500 cm^{-1} region	Characteristic of hydrochloride of tertiary amine
1626 cm^{-1}	C=O stretch
1580 and 1496 cm^{-1}	aromatic rings
846 cm^{-1}	2 adjacent hydrogens on phenyl ring

2.2 Nuclear Magnetic Resonance Spectrum (NMR)

The NMR spectrum of a sample of trimethobenzamide hydrochloride is shown in Figure 2 (2). The spectrum was obtained by dissolving 57.2 mg in 0.5 ml of DMSO- d_6 containing tetramethylsilane as the internal reference. The

Figure 1

Infrared Spectrum of Trimethobenzamide Hydrochloride

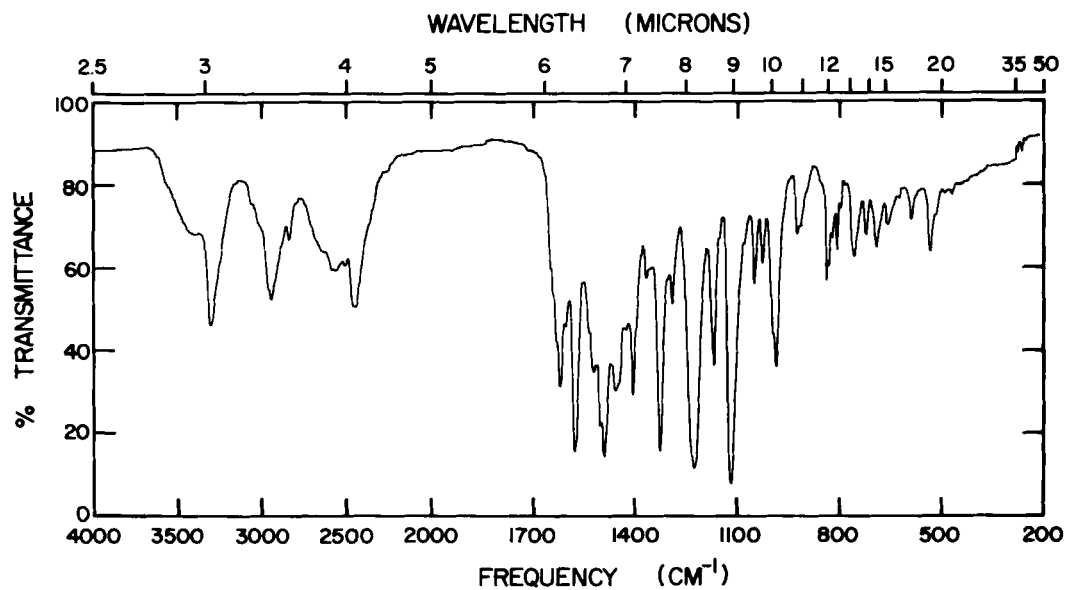
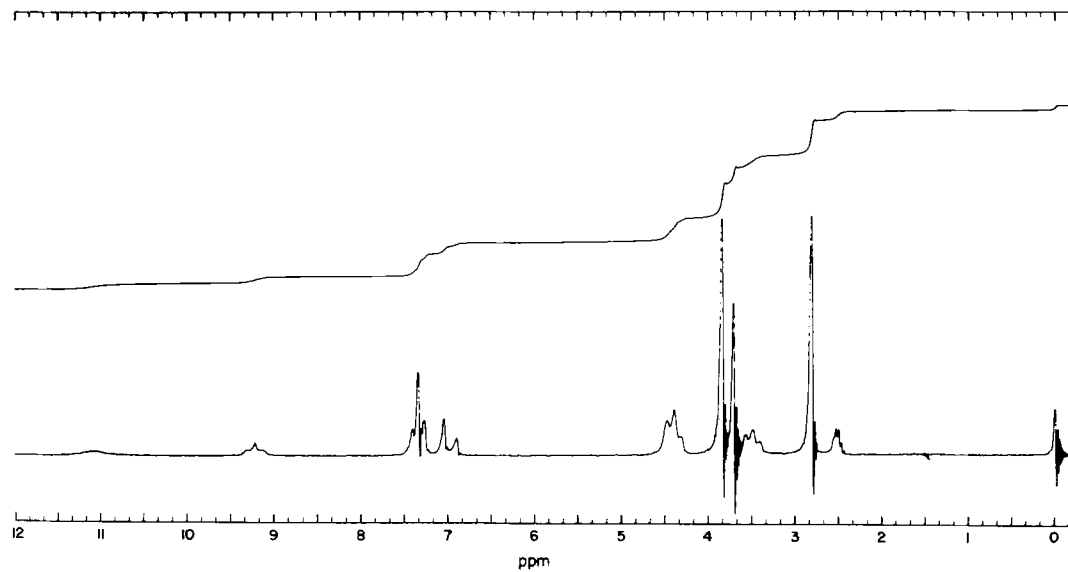


Figure 2

NMR Spectrum of Trimethobenzamide Hydrochloride



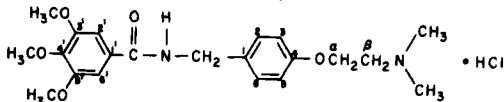
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spectral assignments are given in Table I (2).

Table I

NMR Spectral Assignments for
Trimethobenzamide Hydrochloride*



<u>Protons</u>	<u>No. of Each</u>	<u>Chemical Shift (ppm)</u>	<u>Multiplicity</u>	<u>Coupling Constant</u>
N,N-dimethyl	6	2.83	s	
β -carbon of amino ethoxy group	2	3.48	t	$J_{H_{\beta}-H_{\alpha}} = 5\text{Hz}$
4'-methoxy	3	3.73	s	
3' and 5' methoxy	6	3.85	s	
benzyl hydrogens on C_1	2	4.36	d	
α -carbon of amino ethoxy group	2	approx. 4.3	t	$J_{H_{\alpha}-H_{\beta}} = 5\text{Hz}$
C_2 and C_6 hydrogens	2	6.95	d	$J_{H_6-H_3}, J_{H_2-H_4} = 8\text{Hz}$
C_3 and C_5 hydrogens	2	7.32	d	$J_{H_3-H_6}, J_{H_4-H_2} = 8\text{Hz}$
C_2 , and C_6 , hydrogens	2	7.31	s	
amido hydrogen	1	approx. 9.2	t	
hydrogen of hydrochloride	1	approx. 11.0	s (broad)	

*An arbitrary set of numbers was assigned to the atoms in the structure to simplify the presentation of the data.

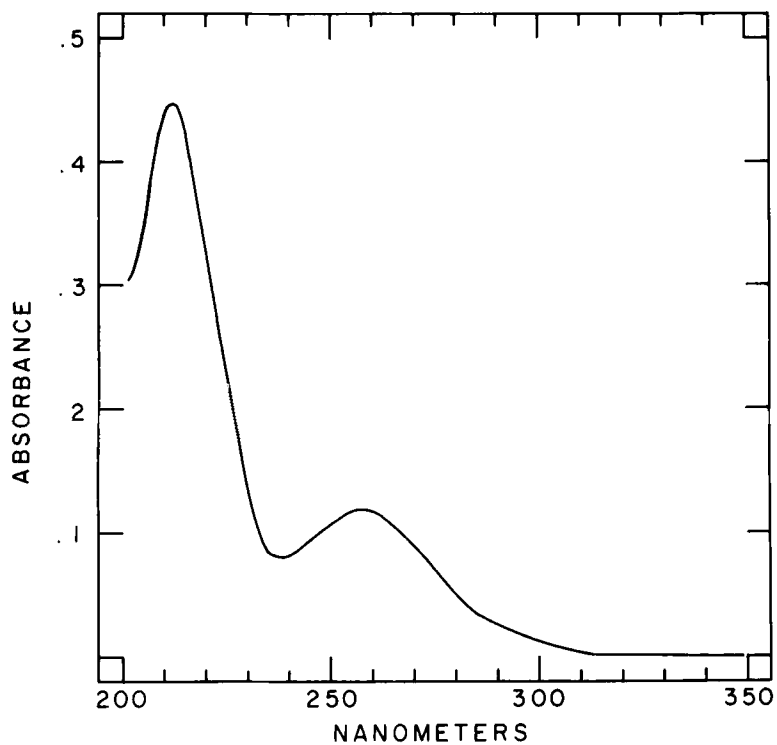
2.3 Ultraviolet Spectrum

The ultraviolet spectrum of trimethobenzamide hydrochloride is shown in Figure 3 (3). The spectrum exhibits two maxima and one minimum in the range of 200—400 nm. The maxima are located at 212 nm ($\epsilon = 4.7 \times 10^4$)

TRIMETHOBENZAMIDE HYDROCHLORIDE

Figure 3

Ultraviolet Spectrum of Trimethobenzamide Hydrochloride



and 258 nm ($\epsilon = 1.2 \times 10^4$), and the minimum is at 238-239 nm. The concentration of trimethobenzamide hydrochloride was 0.404 mg/100 ml of 0.1N HCl.

2.4 Fluorescence Spectrum

The excitation and emission spectra of a sample of reference standard trimethobenzamide hydrochloride are shown in Figure 4 (4). The spectra were recorded on methanol solutions of trimethobenzamide hydrochloride (1 mg/ml) using a Farand MK-1 recording spectrofluorometer. Excitation at 310 nm produced an emission spectrum having a maximum at 341 nm.

2.5 Mass Spectrum

The low resolution mass spectrum of trimethobenzamide is shown in Figure 5 (5). The spectrum was obtained using a CEC 21-110 spectrometer with an ionizing energy of 70eV which was interfaced with a Varian data system 100MS. The data system accepted the output of the spectrometer, calculated the masses, compared their intensities with that of the base peak and plotted the relative intensities as lines of various heights.

The molecular ion was at m/e 388 (free base). The two predominant cleavage products occur at m/e 58, which corresponds to $\text{CH}_2\text{N}(\text{CH}_3)_2$, and m/e 195, corresponding to the trimethoxybenzoyl moiety. Other characteristic masses appearing in the spectrum are m/e 330, which corresponds to the loss of $\text{CH}_2\text{N}(\text{CH}_3)_2$ from the molecular ion, and m/e 317 corresponding to the loss of $\text{C}_4\text{H}_9\text{N}$ from the parent mass. The high resolution spectrum fully confirmed the results of the low resolution scan (5).

2.6 Optical Rotation

Trimethobenzamide hydrochloride exhibits no optical activity.

2.7 Melting Range

The melting range reported in NF XIII is 187-190°C when a Class I procedure is used (6).

2.8 Differential Scanning Calorimetry (DSC)

The DSC curve for a sample of reference standard trimethobenzamide hydrochloride is shown in Figure 6. The instrument used was a Perkin Elmer DSC-1B using a

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Figure 4

Emission and Excitation Spectra
of Trimethobenzamide Hydrochloride

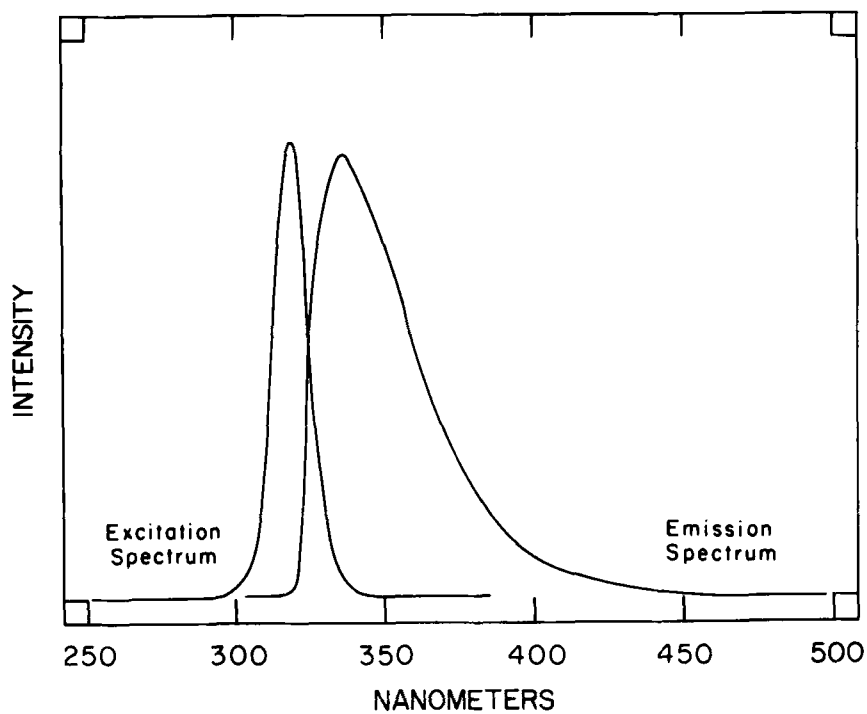
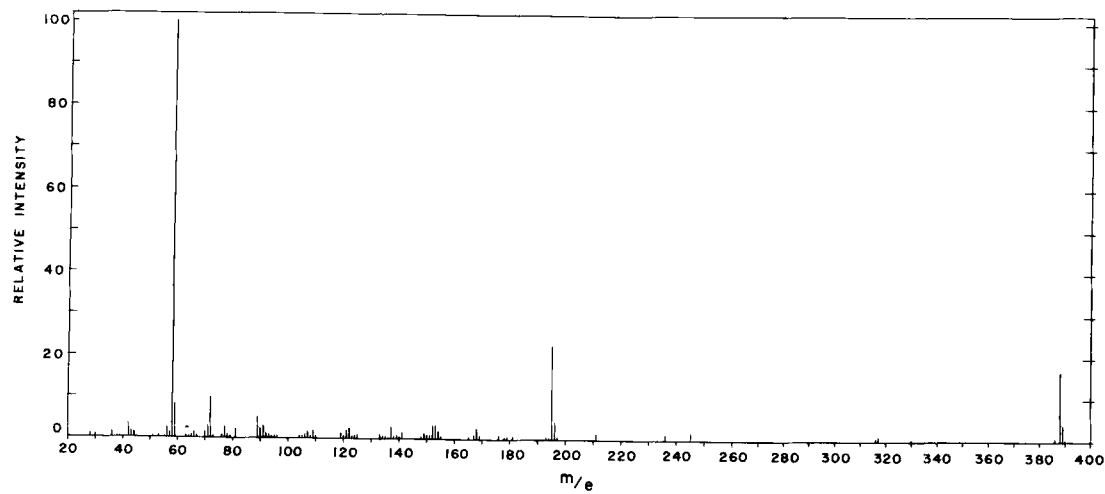


Figure 5

Mass Spectrum of Trimethobenzamide Hydrochloride

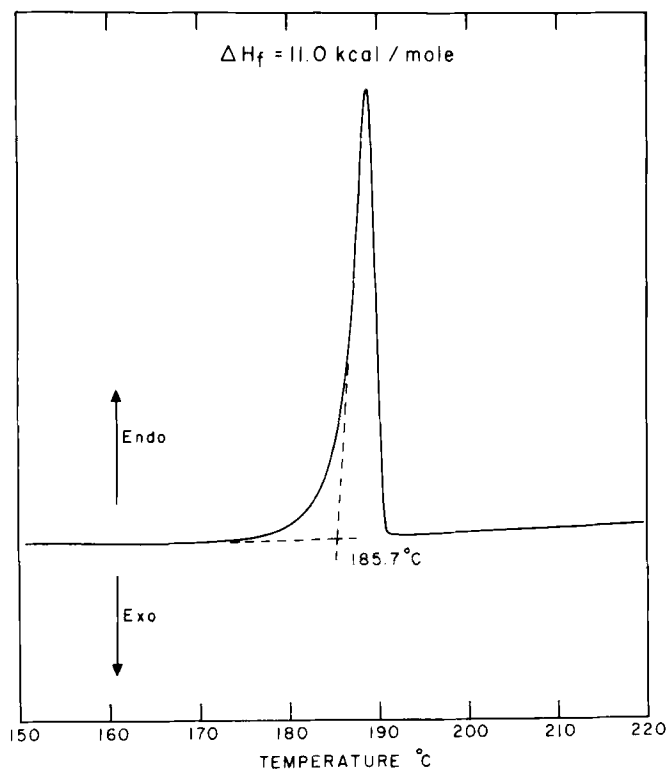


K. W. BLESSEL, B. C. RUDY, AND B. Z. SENKOWSKI

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Figure 6

DSC Curve for Trimethobenzamide Hydrochloride



temperature program of $10^{\circ}\text{C}/\text{min}$. The extrapolated onset of the melting endotherm is 185.8°C with the peak at 188.8°C . All temperatures are corrected. The ΔH_f was calculated to be 11.0 Kcal/mole for the melting endotherm (7).

2.9 Thermogravimetric Analysis (TGA)

The TGA scan of trimethobenzamide hydrochloride showed no significant loss of weight up to 230°C at a heating rate of $10^{\circ}\text{C}/\text{min}$. A continuous loss of weight was observed between 230 – 500°C during which range 80% of the sample weight was lost (7).

2.10 Solubility

The solubility data for a sample of reference standard trimethobenzamide hydrochloride at 25°C is given in Table II (8).

Table II

<u>Solvent</u>	<u>Solubility (mg/ml)</u>
Petroleum Ether (30° – 60°)	0.5
Diethyl Ether	0.8
Water	>500.
2-Propanol	1.7
3A Alcohol	14.1
Chloroform	19.3
95% Ethanol	53.8
Benzene	1.2
Methanol	139.4

2.11 X-ray Crystal Properties

The x-ray powder diffraction data obtained for a sample of reference standard trimethobenzamide hydrochloride are given in Table III (9). The operating conditions of the instrument are given below.

Instrumental Conditions:

General Electric Model XRD-6 Spectrogoniometer

Generator: 50 KV, 12-1/2 MA

Tube target: Copper

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Radiation: Cu K_{α} = 1.542 Å
 Optics: 0.1° Detector slit
 M.R. Soller slit
 3° Beam slit
 0.0007" Ni filter
 4° take off angle
 Goniometer: Scan at 0.2° 2θ per minute
 Detector: Amplifier gain - 16 coarse,
 8.7 fine
 Sealed proportional counter
 tube and DC voltage at plateau
 Pulse height selection E_L -
 5 volts;
 E_U - out
 Rate meter T.C. 4
 2000 C/S full scale
 Recorder: Chart speed 1 inch per 5
 minutes
 Samples: Prepared by grinding at room
 temperature

Table III

Interplanar Spacings from Powder Diffraction Data
 for Trimethobenzamide Hydrochloride

2θ	$d^1(\text{Å})$	I/I_0^2	2θ	$d(\text{Å})^1$	I/I_0^2
4.86	18.2	30	31.13	2.87	8
12.24	7.23	6	31.30	2.86	7
14.26	6.21	75	31.75	2.82	19
14.58	6.08	35	32.50	2.75	29
15.13	5.86	100	34.44	2.60	18
15.70	5.64	19	35.30	2.54	3
16.64	5.33	64	35.56	2.52	3
17.55	5.05	12	36.00	2.49	4
17.86	4.97	12	36.96	2.43	2

$$^1d = (\text{interplanar distance}) \frac{n \lambda}{2 \sin \theta}$$

$^2I/I_0$ = relative intensity (based on highest
 intensity of 100)

18.36	4.83	3	37.46	2.40	2
19.18	4.63	4	37.96	2.37	5
19.49	4.55	25	38.36	2.35	3
20.20	4.40	36	38.94	2.31	7
20.68	4.29	18	39.50	2.28	9
20.96	4.24	14	39.85	2.26	5
21.38	4.16	45	40.46	2.23	6
22.27	3.99	63	41.00	2.20	4
22.63	3.93	34	41.48	2.18	7
23.03	3.86	68	41.96	2.15	3
23.53	3.78	35	42.82	2.11	7
24.66	3.61	45	43.72	2.07	8
25.02	3.56	16	45.00	2.01	4
25.62	3.48	31	45.30	2.00	4
26.12	3.41	57	45.76	1.98	3
26.48	3.37	32	46.18	1.97	4
27.08	3.29	55	46.72	1.94	4
27.86	3.20	10	47.28	1.92	6
28.16	3.17	5	47.54	1.91	4
28.96	3.08	16	48.46	1.88	4
29.96	2.98	31	49.48	1.84	3
30.56	2.93	8	51.15	1.79	7

2.12 Dissociation Constant

The apparent pKa of trimethobenzamide hydrochloride was determined by a potentiometric titration in aqueous solution with 0.02M KOH. The value obtained was 8.27 ± 0.03 (10).

3. Synthesis

The reaction sequence shown in Figure 7 was used to produce ^{14}C -labelled trimethobenzamide hydrochloride and is also one of the synthetic routes to produce this material(11).

4. Stability Degradation

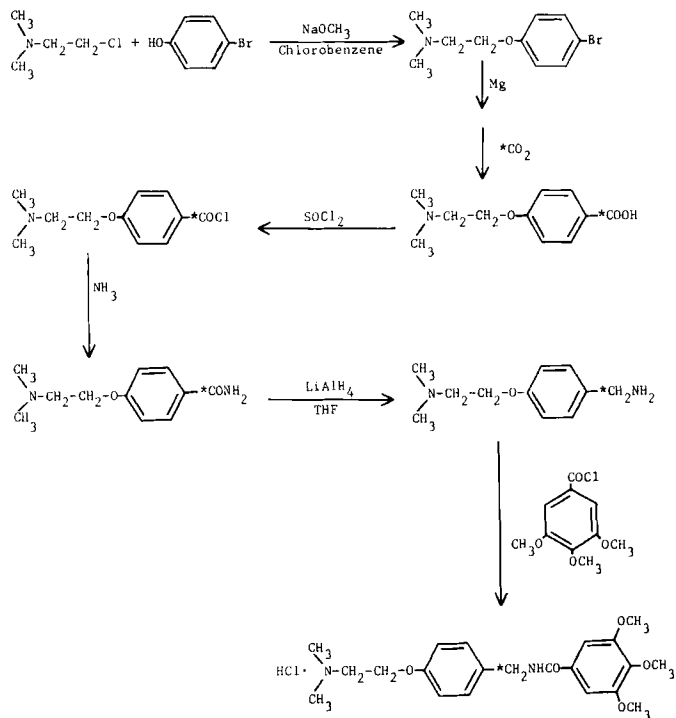
Trimethobenzamide hydrochloride has been shown to be stable when heated at 100°C in acid, alkaline or neutral solution (12). It was also found to be stable when stored at room temperature for a period in excess of five years (13).

5. Drug Metabolic Products

A study of the metabolism of trimethobenzamide hydrochloride in dogs has been carried out utilizing a

Figure 7

Synthesis of ^{14}C -labelled Trimethobenzamide Hydrochloride



*C = ^{14}C

^{14}C -labelled sample of the material (14). It was found that approximately 30% of the dose was excreted intact while biotransformation of part of the remainder gave the mono-N-demethylated and the N-oxide analogs of trimethobenzamide hydrochloride as shown in Figure 8 (14).

6. Methods of Analysis

6.1 Elemental Analysis

The results of an elemental analysis of a reference standard sample of trimethobenzamide hydrochloride are presented in Table IV (15).

Table IV

<u>Element</u>	<u>% Theory</u>	<u>% Found</u>
C	59.36	59.28
H	6.88	6.80
N	6.59	6.66
Cl	8.34	8.34

6.2 Phase Solubility Analysis

Phase solubility analyses have been carried out for trimethobenzamide hydrochloride. An example is shown in Figure 9. The solvent used was absolute ethanol and the equilibration time was 45 hours at 25°C (8).

6.3 Thin Layer Chromatographic Analysis

A thin layer chromatographic method for the separation of trimethobenzamide hydrochloride from its possible metabolites has been developed. The adsorbent was silica gel G and the solvent system was ethyl acetate:ethanol: NH_4OH (90:10:5). The solvent front is allowed to ascend for 15 cm. The plate is then air dried and sprayed with potassium iodoplatinate detecting reagent (16). The approximate R_f values are given in Table V below.

Table V

<u>Compound</u>	<u>R_f</u>
Trimethobenzamide hydrochloride	0.9
mono-N-desmethyl metabolite	0.7
di-N-desmethyl metabolite	0.6

Figure 8
Metabolic Products of Trimethobenzamide Hydrochloride

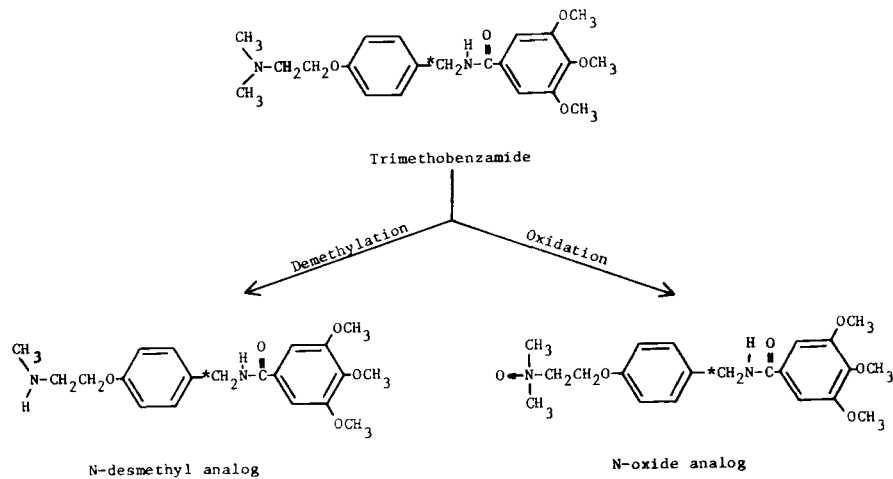
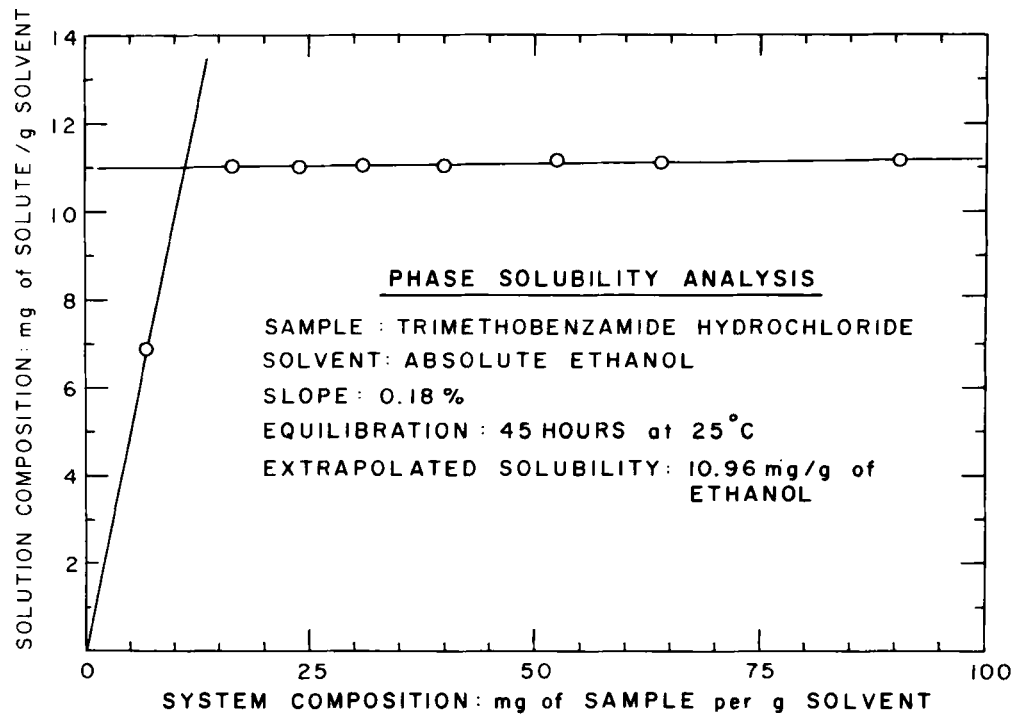


Figure 9



6.4 Direct Spectrophotometric Analysis

Trimethobenzamide hydrochloride may be determined directly in capsules by utilizing the procedure which follows.

An amount of capsule powder equivalent to approximately 50 mg of trimethobenzamide hydrochloride is weighed into a 100 ml volumetric flask. The sample is dissolved and diluted to volume with 0.1N HCl, after which it is filtered. A 4 ml aliquot of the filtrate is transferred to a 100 ml volumetric flask and diluted to volume with 0.1N HCl. The absorbance of the solution is measured at 258 nm using 0.1N HCl as a reference. The amount of trimethobenzamide hydrochloride is calculated by comparison to a sample of the reference standard measured in a similar manner (6).

6.5 Fluorescence Analysis

Trimethobenzamide hydrochloride may be determined by way of the characteristic fluorescence of trimethoxybenzoic acid which is a product of hydrolysis.

The hydrolysis is maximal using 1.5N HCl at 100°C for 17 hours. The liberated trimethoxybenzoic acid is extracted into ether, the ether evaporated and the residue taken up in a pH 2 buffer. The fluorescence is measured at 370 nm and 700 nm using an activating wavelength of 290 nm. The method is applicable to the determination of trimethobenzamide hydrochloride in urine and blood after extraction of the material into chloroform (17).

6.6 Non-Aqueous Titration

The non-aqueous titration of trimethobenzamide hydrochloride with perchloric acid as described in NF XIII is the method of choice for the analysis of the bulk material. The sample is dissolved in glacial acetic acid with the subsequent addition of mercuric acetate T.S. and crystal violet indicator. The titration is performed with 0.1N HClO₄ in glacial acetic acid. Each ml of 0.1N HClO₄ is equivalent to 42.49 mg of thrimethobenzamide hydrochloride (6).

7. Acknowledgement

The authors wish to acknowledge the Research Records Department of Hoffmann-La Roche Inc. for their help in the literature search for this analytical profile.

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ADDENDA

Triamcinolone:

- 6.6. Titrimetric Determination with Lead (IV) - acetate also in the presence of Triamcinolone acetoneide: E. Csizér, S. Görög and T. Szén, Microchimica Acta 1970, 996.
7. Identification by GLC:
B. S. Finkle, E. J. Cherry and D. M. Taylor, J. Chromatog. Sci. 9, 393 (1971).
Plasma levels: M. Kusama, N. Sakauchi, S. Kumaoka Metab. Clin. Exp. 20, 590 (1971).

Triamcinolone Acetonide:

- 6.53. Partition coefficient for n-hexane-chloroform-dioxane water (90:10:40:5) system:
D. J. Weber, T. R. Ennals and H. Mitchner J. Pharm. Sci. 61, 689 (1972).
7. Plasma levels:
M. Kusama, N. Sakauchi, S. Kumaoka Metab. Clin. Exp. 20, 590 (1971).

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ERRATA

Acetohexamide

Vol. 1 p. 7 Fig. 4 TGA spectrum not DTA spectrum.
Fig. 5 DTA spectrum not TGA spectrum.

Erythromycin Estolate

Vol. I p. 103 Erythromycin not Estromycin
(legend under structure).

Halothane

p. 128 line 4: Change formula to:

$$\log_{10} p = 6.8513 - \frac{1082.495}{t+222.44}$$

Levaterenol Bitartrate

Vol. I p. 153, line 8: long not lone

line 12-14: reverse to

$$n_{\alpha} = 1.531 \pm 0.002$$

$$n_{\beta} = 1.577 \pm 0.002$$

$$n_{\gamma} = 1.620 \pm 0.002$$

p. 159, line 13: delete first 169; also
153 (17); not 153 (7)

line 22: delete first 169

line 24-26: Instrument used:

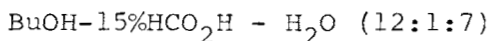
JEOL Model IMS015SC. Electron

beam 75 e.v.

p. 171, line 6: R. K. Kullnig

Nortriptyline Hydrochloride

Vol. I p. 243, line 9 and 10:



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